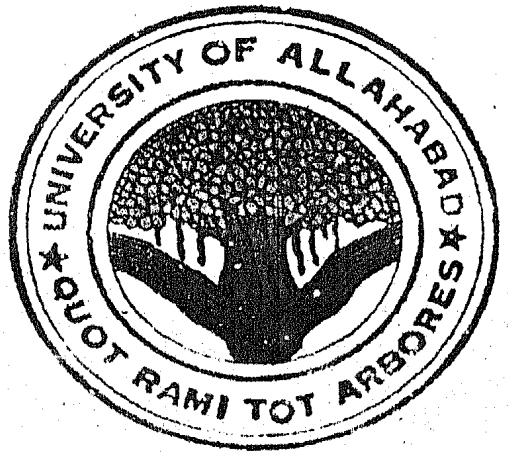
STUDY ON SOME PATHOGENIC FUSARIA



THESIS

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By

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DEDICATED

TO

MY

GRANDFATHER

CERTIFICATE

Certified that the thesis embodies results of original research work and study carried out under my supervision by Mr. Surendra Nath Chaubey M.Sc. (Ag.) Botany.

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PREFACE

The thesis embodies research work on **"Study on some Pathogenic Fusaria"**. The work carried out by me from January 2000 to November 2002 in the Someshwer Nath Bhargva Agricultural Botany Laboratory, Botany Department, University of Allahabad and a comprehensive survey of Allahabad and its adjacent regions was made.

The thesis embodies researches with studies on some Fusaria isolated from various crop fields, wilted trees and seeds of crop plants.

The thesis is compiled into Eleven Chapters. First chapter deals with Introduction followed by Materials and Methods (Chapter 2). Isolation and Pathological Studies are discussed in Chapter 3. Morphological Studies of *Fusarium* is given in Chapter 4. The Chapter 5 includes Diseases caused by Pathogenic Fusaria. Chapter 6 contains, Toxin produced by Fusaria. Survival Studies are given in Chapter 7. Biological Control is discussed in Chapter 8. Discussion is given in Chapter 9. The whole work is summarized in Chapter 10 as Summary. The bibliography containing the references cited in the text is appended in the end (Chapter 11). Abstract of the present work is also submitted along with the thesis separately.

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INTRODUCTION

I see you stand like greyhounds in the slips, straining upon the start. The game's afoot : Follow your spirit, and upon this charge cry "God for Harry ! Eng'and and Saint George !"

— William Shakespeare

The genus *Fusarium* may have been one of the earlier fungi to become established on earth. The genus *Fusarium* was erected by Link (1809) for species with fusiform, nonseptate spores borne on a stroma and was based on *Fusarium roseum*. Wollenweber and Reinking (1935) stated that of the two original specimens still existing one was *Fusarium sambucinum* and the other *F. graminearum*. In terms of the International Botanical Code the genus was validated by Fries (1821) who included it in his order Tuberculariae. With the development of pure culture methods for *Fusarium* identification, the presence of a stroma or sporodochium was no longer accepted as an essential character of the genus. The presence of fusoid macroconidia with a foot cell bearing some kind of heel is now accepted as the most definite character and this foot cell separates it from *Cylindrocarpon*, the most closely related genus. Microconidia and terminal or intercalary chlamydospores may be present or absent. Perithecial states, where known, belong to the Hypocreales.

Fusarium species are widely distributed in soil and on organic substrates and have been isolated from permafrost in the arctic and from the sand of the Sahara. They abound in cultivated soil both in temperate and tropical regions and are amongst the fungi most frequently isolated by the plant pathologist. *Fusaria* are also involved in diseases of animals and man, and as major storage rots often produce toxins which contaminate human and animal food. As with many soil fungi they are abundantly endowed with means of survival, one of the mechanisms of which is the capacity for rapid change, often morphologically as well as physiologically, to a new environment. Thus they can survive on a wide range of substrates and have been isolated from many preserved foods, from stored chemicals and along with *Cladosporium resinae* from aircraft fuel tanks.

The predominant interest in the genus has been and still is in their role as plant pathogens. The serious wilts, such as Panama disease of bananas caused by *F. oxysporum*, are amongst the most devastating plant diseases in the world. *F. moniliforme, F. graminearum, F. avenaceum* and *F. culmorum* are serious pathogens of Gramineae causing pokkah-boeng of sugarcane, bakanae disease of rice, pre-and post-emergence blight of cereals and many others. Strains of *F. solani* are also of world-wide occurrence as root rots, but may also cause cankers of hardwood trees.

Because of their capacity for rapid change, species identification presents certain problems, some more imaginary than real; but it is this reputation of

complexity and confusion that has made many plant pathologists approach their identification with caution or avoid them altogether. There is certainly no need for such hesitation so long as two principal rules are followed. As with *Penicillium, Aspergillus* and several other genera of the fungi imperfecti. *Fusarium* species in general can only be identified in pure culture and preferably from cultures started from single spores. It matters little whether these are single macroconidia or microconidia as both begin initially with a single nucleus. Secondly, as *Fusarium* isolates have a remarkable facility for adapting both their form and colour in response to the pressure of the cultural environment the culture medium must be standardised and kept uniform for identification purposes. A mycologist or plant pathologist may use whatever medium he wishes but it is necessary to know the response of different *Fusarium* isolates to those conditions. For the purpose of using this book the description of the cultures is based on growth on potato sucrose agar adjusted if necessary to pH 6.5-7. Descriptions are based on cultures started from single conidia unless otherwise stated and growth rate is given as the average diameter attained by a series of single conidia isolates after four days grown at 22-25°C, 10-14 inches below a daylight fluorescent tube on a 12 hr on/off sequence. For purposes of examination and measurement mycelium and spores were mounted in erythrosin in 10% ammonium hydroxide and the coverslip sealed with two layers of nail varnish.

During the past 38 years since the work of Mason (1933), in fact since Vuillemin (1911), the sporogenous or conidiogenous cell has gradually become the most dominant single character in the classification of the fungi imperfecti. It is to say the least singularly odd, therefore, that the conidiogenous cell has never been utilised in the classification of *Fusarium* species. It has of course been assumed that *Fusarium* spores are phialospores and there is nothing more to be said. The fact that phialospores and blastospores occur within the genus has not previously been stressed although blastosporic conidiogenous cells were illustrated both by Seemuller (1968) and Messiaen & Cassini (1968). Even in species with only phialidic conidiogenous cells the structure is often characteristic of the species. Thus the microconidiophore structure provides the easiest means of separating *F. oxysporum* from *F. solani* and the polyphialides of *F. moniliforme* var. *subglutinans* readily separate it from *F. oxysporum* without having to wait for chlamydospore development. The blastospores of *F. sporotrichioides* readily separate this species from *F. tricinctum*.

Fusarium structures resembling chlamydospores in coal which was estimated to date back 200 million years. But certainly if any fungus were to emigrate to another planet. *Fusarium*, as a most adaptable, versatile and pioneering genus, would be a good candidate for establishment out there. Some of its species do well with very little oxygen or water and with unusual mixtures of gases and chemicals, and flourish under extremes of environmental stress.

Fusarium is well adapted to habitation on this earth and is present in our soil, water, air and organic materials. Perhaps more is known about the genus *Fusarium* and its species than is known about other genera of fungi. Yet, the more we learn the more we realize how little we actually known of the genus which Link described 169 years ago.

The importance of *Fusarium* to world food production has greatly increased in recent decades. For example, the number of known wilt diseases caused by *formae speciales* of *F. oxysporum* has increased from 24 Snyder (1940) in 1940 to 76 as listed by Booth (1971). The worldwide significance of the destructiveness of *Fusarium* diseases of wheat, barley and corn has become apparent to several *Fusarium* workers in the past 10 years. Even *Fusarium nivale*, once thought to be economically important only in northern countries, is now recognized as important in some southern regions such as Sicily Piglionica et. al. (1974) and Africa. That new *formae speciales* of *F. solani* have been described as having a role in severe infections of human and animal eyes, is causing increasing concern.

Whether the greater awareness of *Fusarium* pathogens in today's world stems from greater recognition by more *Fusarium* workers, or from the origination of new pathogenic forms, or both, the fact remains that the genus has emerged as a leading threat to most of the principal food crops of the world, as well as to oil, fiber, ornamental and forest plantings.

We have the anomaly in the ascomycetes, that if an imperfect fungus reveals its perfect state as part of its complete life cycle, then that state may be given a new genus and species name. For example, after the perfect state of *F. solani* was discovered, it was later named *Nectria haematococca* instead of *Hypomyces solani* as Wollenweber chose.

Thus if *F. solani,* with its mycelium and three kinds of vegetative spores, wishes, so to speak, to keep its specific identity, then it must never indulge in its inherent sexual phase. For as soon as the sexual stage appears-contraceptives for fungi were not in vogue at that time-with the formation of beautiful red or white perithecia, the fungus suddenly becomes a different genus and species.

Fusarium provides an ideal genus for the experimental study of biology as expressed genetically and environmentally. An individual is usually haploid in its vegetative state. The cells are hermaphroditic but not heterocaryotic. It may be either homothallic or heterothallic when it passes briefly through a diploid phase. Its physiologic or biochemical behaviour may or may not be evident in its morphologic or cultivar appearance, yet it may be a powerful and economically dangerous organism.

The population of *Fusarium* in agricultural fields is often as high as 100,000 propagules per gram of soil, or more, as shown by Smith (1971). Most if not all of these clones may be beneficial saprophytes, aggressively decomposing

dead organic matter of many kinds. Where a pathogen has been introduced it may reach populations of 4,000 or 5,000 propagules per gram of soil in the case of *F. solani* f. sp. *phaseoli*, or 10,000 or more for *F. roseum* f. sp. *cerealis* and variously for formae speciales of *F. oxysporum*, yet pathogens usually represent only a small percentage of the total *Fusarium* count. To have meaning, a soil count must be combined with proper pathogenicity tests to determine which clones of the *Fusarium* are pathogens.

Air dispersal of *F. moniliforme* by ascospores of the *Gibberella* state on rice may reach very high levels as shown by Sun, (1975), Yu and Sun, (1976). The genetic variability of this heterothallic pathogen increases its threat as a pathogen in many parts of the world. Likewise, the air-borne spread of *F. nivale* by its *Calonectria* state, and of *F. roseum* f. sp. *cerealis* by its *Gibberella* state, is dramatically destructive in many regions of the world where corn, wheat, barley or rice are principle crops. Water-borne macroconidia disperse pathogens of the species of *F. solani* and of *F. oxysporum*, while insects transport the microconidia of *F. rigidiuscula* and *F. moniliforme*. Man further aids in the spread of *Fusarium* pathogens in his distribution of infected or infested seeds or other planting materials and by cultural practices.

It seems evident, then, that *Fusarium* is a growing threat to the world crops, more serious now with the shortages of food than at any time before. Losses from

the spread of the date palm Bayoud disease have already turned a former date-exporting nation into a date-importing one.

An alternative is for us to begin informing governments and granting agencies now of the losses due to *Fusarium* in many crops in most countries, and of the danger of eating or feeding infected products. We are in a better position today than ever before to mount a coordinated attack on *Fusarium* pathogens in spite of our lack of adequate support. We know something about the pathogens, if any, of each species.

We now recognize the role of water stress as demonstrated by Cook et al. (1970) and something of the different epidemiologies of the diseases in different climates and regions of the world. We have an illustrated guide to *Fusarium* species by Toussoun and Nelson (1976) and we have books with much assembled information and guides Messiaen (1959), Synder (1965).

The *Fusaria* are widely distributed in soil, on subterranean and aerial plant parts, plant debris, and other organic substrates Wollenweber et al. (1935), Booth (1971), Gordon (1960), Wellman (1972). They are common in tropical and temperate regions and are also found in desert, alpine, and arctic areas, where harsh climatic conditions prevail Booth (1971), Burgess et al. (1975), Francis et al. (1975). Many species occur abundantly in fertile cultivated and rangeland soils but are relatively uncommon in forest soils Lim et al., (1970), Snyder et al., (1968). A

few representatives are regarded as entomogenous fungi Booth (1971), Gordon (1956), and several others are reported to be fungal parasites Booth (1971). The *Fusaria* are often regarded as soil-borne fungi because of their abundance in soil and their frequent association with plant roots, either as parasites or saprophytes. However, many have active or passive means of dispersal in the atmosphere and are common colonizers of aerial plant parts, where they may result in diseases of considerable economic importance Bolkan et al. (1979), Booth (1971), Francis et al. (1975). Some of these air-borne *Fusaria* are rarely encountered in isolations from soil or roots. The widespread distribution of most *Fusarium* populations can probably be attributed to two key factors, their ability to colonize a wide range of substrates and their efficient mechanisms for dispersal in time and/or space. These two factors also contribute to their ability to readily adapt to new ecological niches created by man.

The majority of the *Fusaria* are normally found in or on soil, where they exist as colonizers of living plant parts or plant residues within the soil or adjacent to the soil surface, normally within 100 cm Booth (1971), Burgess et al. (1975), Francis et al. (1975), Gordon (1956). In the absence of a suitable substrate, these fungi persist as resistant (dormant) hyphae in plant residues colonized parasitically or saprophytically, or as discrete propagules such as chlamydospores and resistant conidia. Traditionally these populations have been regarded as soil-borne fungi, although soil-based would seem a more appropriate term. Typical representatives

are *Fusarium oxysporum, F. solani, F. roseum 'Culmorum, F. roseum* 'Graminearum' Group Francis (1977), and *F. roseum 'equiseti'*.

A number of *Fusaria* normally exist as primary or secondary colonizers of aerial plant parts Booth (1971), Siddiqi et al., (1963), Wollman (1972). These fit the traditional definition of air-borne fungi. They each have at least one efficient method of air-dispersal, viz. passive wind-borne or rain-splash dispersal or active dispersal by ascospores. In the absence of suitable substrates they persist as resistant hyphae or conidia, or as immature perithecia in dead twigs and branches attached to healthy branches in the plant canopy or in residues on the soil surface Booth (1971), Wearing et al., (1978). This mode of existence is characteristic of such populations as *F. rigidiusculum, F. lateritium,* and *F. roseum* 'Graminearum' Group 2.

Fusaria are abundant in soil and commonly associated with roots and the lower parts of plants, they are also encountered on aerial plant parts. Their presence on aerial parts is not surprising and is not different from the behaviour of many other soil-based genera. It can be attributed to their passive dispersal in the atmosphere Gregory et al. (1959), Hirst (1965), their ability to utilise a wide range of substrates, and in some instances, abnormal environmental conditions Francis et al. (1977). The fact that these fungi can be dispersed in the atmosphere is often overlooked and is not well documented Hirst (1965), Horst et al. (1970). The soil-borne *Fusaria* often form sporodochia on substrates on or just above the soil

surface, and the spores in these fruiting bodies can be dispersed by rain splash directly or after they are washed onto the soil surface Gregory et al. (1959), Hirst (1965). Conidia in sporodochia are probably not suited to dispersal by wind in dry weather because they are usually cemented to the substrate Hirst (1965). It has also been suggested that air dispersal of these *Fusaria* may be associated with dust particles Burkholder (1919), Horst (1970), and Ooka and Kommedahl Ooka et al., (1977) have presented evidence of long-distance dispersal in dust storms over several hundred kilometres. It is probably that these fungi will also be common in the atmosphere where soil is being cultivated or crops are being harvested. The role of insects in aerial dispersal of soil-borne *Fusaria* has not been studied in detail but is probably significant in some circumstances Booth (1971), Burkholder (1919). Since these fungi are present in the atmosphere, some of their inocula will be deposited on aerial plant surfaces where they may remain inactive or, given suitable conditions, germinate and colonize the substratum or part thereof. Such colonization may be parasitic or saprophytic in nature.

Fusarium oxysporum is one of the most common soil-borne *Fusaria* Booth (1971), Gordon (1960). Some intra-specific populations of this highly variable fungus are very pathogenic, causing wilts and crown and root rots, while others appear to be vigorous saprophytes which are common colonizers of senescent or damaged plant tissue Armstrong et al. (1975), Booth (1971). It is one of the most common *Fusaria* isolated from roots and crowns and is active under a wide range

of environmental conditions Burgess et al. (1975). This species readily forms chlamydospores and so is well adapted to long-term survival in soil Messiaen et al. (1965). *Fusarium oxysporum* can also persist as hyphae in organic residues Booth (1971). Thus pathogenic members of this species are particularly difficult to eliminate from infested soil by rotation or long fallow. Elimination of some of the wilt *Fusaria* is rendered more difficult by their ability to colonize non-susceptible species Armstrong et al., (1948). Although *F. oxysporum* has been isolated from the air Rowe et al., (1977), it is not well adapted to air dispersal. Its presence in the atmosphere can probably be attributed to wind-blown soil or organic debris Ooka et al., (1977). Abundant sporodochia are often formed on the stems of plants affected by *Fusarium* wilt and these would provide a source of inoculum for localised dispersal by rain splash. *Fusarium oxysporum* is not known to form perithecia. I am not aware of any reports of its association with localised diseases of aerial plant parts and none are listed by Booth (1971). Air dispersal may, however, be important in the ecology of the fungus in some situations. For example Rowe et al. (1977) have produced evidence that air-borne microconidia of *F. oxysporum* f. sp. *radicis-lycopersici,* which causes crown and root rot of tomato Jarvis et al., (1978) can bring about re-colonization of steamed soil in greenhouses. The sources of the air-borne microconidia were presumed to be infested tomato-plant residues outside the greenhouses.

In temperate areas *F. solani* exists as a common soil-borne fungus Booth (1971), Gordon (1956), Kommedahl et al., (1973), which includes both saprophytes and parasites Snyder et al., (1941). Some parasitic members are important root and crown rot pathogens Booth (1971), Burke (1965), while others have been implicated as canker-causing organisms of several hardwood trees Booth (1971). In the absence of suitable substrates the fungus survives primarily as chlamydospores, the importance of which was first suggested by Burkholder (1919) and later confirmed by Nash et al. (1961) and others Messiaen et al., (1965). Data from studies by Schroth and Hendrix (1962) suggest that survival of *F. solani* f. sp. *phaseoli* in agricultural land is enhanced by temporary supplies of nutrients in diffusates from non-susceptible plants and crop residues which enable the fungus to vegetate and form new chlamydospores. It is highly probable that the survival of other members of *F. solani* is also enhanced by diffusates from some plant and crop residues. *Fusarium solani* has occasionally been isolated from air in temperate areas, where its presence is probably a result of wind-blown soil or organic debris Horst et al., (1970), or rain-splashed conidia from sporodochia Gregory et al., (1959), but perithecia are rarely encountered in temperate areas, where *F. solani* is generally not a common colonizer of aerial plant parts Booth (1971).

Fusarium roseum '*equiseti*' is extremely common in and on soil, where it exists as a igorous saprophyte and survives as chlamydospores or mycelial

fragments either free in the soil matrix or in organic residues in or on soil. Although it normally exists as a saprophyte, *F. roseum 'equiseti'* has occasionally been implicated in disease problems Joffe et al., (1967). It is a common colonizer of senescing wheat and maize tissues, from which it can be readily isolated Francis et al., (1975).

Air-borne *Fusaria* are colonizers of aerial plant parts and are adapted to dispersal in the atmosphere by passive or active means. They are rarely encountered in soil. Many representatives of this group are important pathogens causing, for example, twig blights and cankers, stem cankers, collar rots, and inflorescence blights. Rain-splash of conidia constitutes the main means of passive dispersal, although insects probably have a role in some situations. Active dispersal of ascospores provides a major means of dispersal for those members which form abundant perithecia in nature. These fungi survive on infested plant parts that may either remain attached to the plant or fall onto the soil surface. They are rarely isolated from soil.

Fusaria are also common colonizers of subterranean plant parts and residues in soil but also of aerial plant parts and residues on the soil surface. Traditionally they have been regarded as soil-borne fungi.

A typical representative of this category is *F. moniliforme*, a species which is widely distributed in tropical and temperate regions Booth (1971), Francis et al.

(1975), Gordon (1960). It is common in soil and has been isolated from the roots of members of the Gramineae, Leguminoseae, and other families. *Fusarium moniliforme* is also commonly encountered as a primary or secondary colonizer of aerial plant parts. It is, for example, responsible for stalk rots of maize and sorghum, head rots, stem and top rot of sugar cane ('pokkah boeng' disease), and rot of banana flowers and fruit Booth (1971), Wellman (1972). It has been commonly isolated from cultivated and range land soils Gordon (1956). Apparently the fungus survives as mycelium in plant residues in soil or on the soil surface Messiaen et al., (1965), Nyvall et al., (1968). Chlamydospore formation by *F. moniliforme* has not been observed in culture Booth (1971) or soil Messiaen et al., (1965).

Fusarium moniliforme is well adapted for dispersal in the atmosphere in dry or wet weather as it forms macroconidia is sporodochia and chains of microconidia in dry powdery masses. Ooka and Kommedahl (1977), in studies on its dispersal in maize fields, concluded that conidia can be dislodged and dispersed by wind or by rain-splash but they did not identify the type of conidia involved. Presumably the microconidia are predominantly involved in wind disperal, whereas both microconidia and macroconidia could be dispersed by rain-splash. Ooka and Kommedahl (1977) also suggested that the fungus could be dispersed in wind-blown soil and fragments of infested crop residues. Although this fungus is reported to produce perithecia on dead plant material Booth (1971).

Fusarium moniliforme 'Subglutinans' can also be included in this category. It has regularly been isolated from soil Wearing et al., (1977), and is a common colonizer of aerial plant parts Francis et al., (1975), Kuhlman et al., (1978). It has, for example, long been recognised as a cause of cob and grain rot of maize Booth (1971), Edwards (1936). Recent studies have also shown it to be the cause of pitch canker of southern pines in the United States Kuhlman et al., (1978) and fruit rot of pineapple in Brazil Balkan et al., (1979). Infection of aerial plant parts presumably results from conidia dispersed in the air. However, *F. moniliforme* 'Subglutinans' is known to produce perithecia in nature Booth (1971), so ascospores by aid dispersal in some environments. There have been no studies on the mode of dispersal of the conidia produced by this fungus, but it does form abundant macroconidia sporodochia and microconidia in false-heads. Rain-splash of these spores would provide efficient means of localised air dispersal.

In defining the mode of existence of a particular *Fusarium* it must be borne in mind that it may consist of two or more morphologically similar populations which differ in respect to their modes of existence and physiological attributes. For example, two populations designated Groups 1 and 2 have been shown to exist within *F. roseum* 'Graminearum' Francis et al., (1977). They are distinguished by the presence or absence of perithecia in culture. Representatives of these two populations form similar macroconidia in culture but produce slightly different colonies, both in respect to morphology and growth rate, on potato-dextrose agar.

Members of Group 1 do not form perithecia in culture, do so very rarely in nature, and are normally associated with crown and root rots of cereals and grasses Burgess et al., (1975), Francis et al., (1977). In contrast, members of Group 2 form perithecia abundantly in culture and in nature and are normally associated with diseases of aerial plant parts, both as primary and secondary colonizers Francis et al., (1977). They are responsible for diseases such as head scab of wheat, barley and oats; stalk and cob rot of maize and pearl millet; and stub-dieback of carnations Francis et al., (1975).

Members of Group 1 survive as hyphae in residue in and on soil for at least one to two years under certain environmental conditions Burgess et al., (1968). They do not produce chlamydospores in soil Wearing et al., (1977). Presumably infection of plants by members of Group 1 is a result of direct contact between the plant parts and infested residue. However, the fungus can form abundant sporodochia on leaf-sheath tissue of cereals and grasses affected by crown rot under wet conditions. It is throught that rain-splash dispersal of these spores provides the source of inoculum which results in occasional infection of the inflorescences of cereals and grasses (head scab) by members of Group 1 Francis et al., (1977).

Members of Group 2 survive as perithecia and hyphae on and in residues, respectively Wearing et al., (1978). Under suitable conditions the perithecia actively discharge ascospores, which are throught to act as the main source of

inoculum for infection of aerial plant parts. A limited amount of infection probably results from rain-splashed macroconidia or by direct contact with infested residue.

Although the two populations probably occur in most areas, one is usually more abundant because it is favoured by the prevailing environmental conditions and/or the presence of particular hosts Francis et al., (1977). Thus members of Group 1 are usually more common in drier areas, possibly because dry conditions favour the survival of this fungus in residue Burgess et al., (1968). Crown rot of wheat and other grasses caused by members of Group 1 is more severe when infected plants are affected by dry conditions. This factor may also favour an increase in the incidence of the fungus. However, it is known that symptomless wheat plants can be extensively colonized by members of Group 1 under optimal soil moisture conditions Taylor et al., (1980).

Members of Group 2 tend to be more common in areas where it is warm and humid for at least part of the year when suitable substrates are available Francis et al., (1975). Warm humid conditions favour the formation of perithecia and ascospore discharge Tschanz et al., (1976) and hence infection and colonization of aerial plant parts.

Seedling blight occurs in both types of environment. In humid regions it is most probably the result of seed-borne inoculum of Group 2 on or in seed from heads affected by scab or rot. In dry areas seedling death is less common but can

occur in drought periods and is normally caused by soil-borne inoculum of Group1.

Survey data and experimental results indicate that the relative abundance, and range, of *Fusaria* isolated from soil can be related to climate, Lim et al., (1970), vegetation Snyder et al., (1968), soil moisture Lim (1967), Stover (1953), soil type Stotsky et al., (1963) and fertility Snyder et al., (1968). The frequency of isolation and range of *Fusaria* isolated from plants can also be correlated with environmental factors Booth (1971), Burgess (1975). Thus it is not surprising that man's activities influence the relative abundance and the range of *Fusaria* present at a particular location. His activities affect the *Fusaria* through changes in the nature and availability of substrates, their influence on dispersal in time and space, and through modification of the environment.

Changes in the nature and availability of substrate occur whenever a pasture or crop rotation is altered. Snyder and Nash (1968) for example, found that *F. roseum* 'Culmorum' was extremely common in long-term cereal plots at Rothamsted in England, whereas it was quite rare in long-term plots planted to broad-leaf crops in the same locality. All plots were cultivated. The use of resistant cultivars of a particular host crop may also lead to changes in the population of the relevant pathogen. Such changes have been well documented in respect to the wilt *Fusaria* Armstrong et al., (1975).

The influence of cultivation on *Fusaria* in undisturbed grassland or forest soils is difficult to assess because it is usually accompanied by a dramatic change in the nature and availability of substrate and the use of fertilizers. Comparisons of *Fusaria* in cultivated and adjacent undisturbed soils indicate that the cultivation and cropping of soils does have a significant effect on the abundance of *Fusaria* in soil, and in some instances on the range of populations which can be recovered Lim et al., (1970). For example, found that the recovery of *Fusaria* from undisturbed prairie soils differed significantly from the recovery from similar soils planted to maize. Their results indicated that cultivation and maize production resulted in a significant increase in the abundance of *F. roseum* and *F. moniliforme*, whereas other species were generally not affected.

Changes in the abundance of *Fusaria* which follow the clearing of forests or woodlands can be quite dramatic. This is not surprising as survey data indicate that the *Fusaria* are common in cultivated soils but much less common in forest and woodland soils Lim et al., (1970). An appropriate example is provided by the influence of cultivation, cropping, and pasture production on the Atherton Plateau in north Queensland, Australia. This plateau is in the tropics and prior to settlement in the last century was covered by rainforest. The *Fusaria* are common in the cultivated and pasture soils of the plateau. *Fusarium roseum* 'Graminearum' Group 2 is extremely common as a colonizer of maize stalks, senescing grass stems, and plant residues on the soil surface.

The dispersal of the *Fusaria* in space can be affected by man's activities, thus changing the distribution patterns of these fungi. Dispersal can be enhanced through the transport of contaminated seed Booth (1971), Edwards (1936), soil Booth (1971), propagating materials, and dust caused by cultivation Burkhalder (1919), Horst et al., (1970). Such effects will probably have a negligible impact on the occurrence of the cosmopolitan *Fusaria* but could be very important in the successful dissemination of some pathogens which are not widely distributed.

The use of fertilizers, especially nitrogen, has also been correlated with frequency of isolation of the *Fusaria* from soil and plants Snyder et al., (1968), Warren et al., (1973). Studies by Butler (1959) also suggest that the nitrogen status of soil may influence saprophytic persistence. Further studies on the influence of soil nitrogen on survival are warranted.

The above examples provide a brief summary of some of man's activities which can have a profound influence on the distribution and abundance of the *Fusaria* and which in some instances can result in their establishment in areas where the environment would normally limit their activity.

The greatest number of fungal wilt diseases known in higher plants are caused by species of the genera *Fusarium* and Verticillium. However, other genera of fungi cause vascular wilts, especially in deciduous, woody perennials. For example, in the genus Ceratocystis, both the highly destructive Dutch elm disease

is caused by *C. ulmi*, and oak wilt is caused by *C. fagacearum*. Two other species of this genus, *C. fimbriata.* f. *platani* and *C. coerulescens*,, cause the London plane tree disease and sapstreak of sugar maple and tulip tree, respectively. However, these two diseases are usually excluded as typical vascular wilts because the pathogen is not limited strictly to the vascular tissue in the early stages of the diseases.

Other fungi causing wilt diseases include *Cephalosporium diospyri,* incitant of persimmon wilt, Crandell, (1945) and the decline of oak species and elm in east Texas, Van Arsdel, (1972), and *Dothiorella (Cephalosporium) ulmi*, the causal organism of a wilt of American elm, *Ulmus americana*, Verrall and May, (1937).

In plant pathology, the concept of toxin is not limited to one group of compound. All kinds of substances, produced by the pathogen, which are capable of reproducing symptoms similar to that found in natural infections, are toxin.

On the basis of the number of different economic plant species involved, the wilt diseases caused by species of the genera *Fusarium* is by far the most numerous. During the period from 1886 to 1925, for example, *Fusarium* wilt diseases were reported on such crops as tomato, Massee, (1895), crucifers, Smith, (1899), peas, Linford, (1928), watermelons, Smith, (1899), bananas, Brandes, (1918) and numerous others.

As pathogenic fungi *Fusarium* sp. cause heavy damage to crop plants and trees. It is also due to lack of apppropriate control measured. It was therefore, decided to undertake studies on some pathogenic *Fusaria.*

MATERIALS AND METHODS

"It is common sense to take a method and try it : If it fails, admit a frankly and try another".

— Franklin D. Roosevelt

Isolation From Soil

Fusarium species occur widely in the soil both as mycelium and chlamydospores amongst the soil particles or plant debris. Isolation from soil may be complicated by the presence of abundant mould spores although most *Fusarium* species have strong competitive ability coupled with rapid growth. Therefore on a weak dilution plate or by scattering soil particles on the dried surface of Tapwater Agar (medium 4 below) individual colonies can usually be isolated before they are contaminated by adjacent species.

Nash & Snyder (1962) outlined methods of sampling soil for *Fusarium* and devised a special medium, Nash & Snyder (1962) peptone PCNB Medium for the direct isolation of *Fusarium* species from freshly collected soil. Best results were obtained by allowing the poured Petri-dishes to dry in a cool, dark place for 3-5 days before spreading the diluted soil suspension over the agar surface. This drying reduces the ability of bacteria, yeasts and moulds to spread out over the

surface and infect the *Fusarium* colonies as the liquid from the spore suspension is quickly absorbed by the dried agar. Petri-dishes were then incubated for the first few days in diffuse daylight which delayed the development of phycomycetous species. Papavizas (1967) evaluated the various media and antimicrobial agents that have been recommended for the isolation of *Fusarium* species from soil. He summarised their value with regard to their general effectiveness and simplicity of preparation and recommended the use of a modified form of peptone-PCNB (quintozene) (6).

Isolation from Infected Parts

Necrotic areas following wilt, cankers and branch tips suffering from dieback often form mycelium, conidiophores or sporodochia, and isolations can be made directly from these. *Fusarium* species penetrating the vascular tissue of roots and stems can best be isolated following surface sterilisation by mercuric chloride or sodium hypochlorite for 1-5 min of a small piece of the material. After surface sterilisation the material is washed, a small piece is sliced off each end so that residual sterilant is not carried over, and the centre piece is placed on a suitable agar plate. After 24-36 hr at 25°C (18°C for suspected *F. nivale*) the *Fusarium* mycelium should have grown out sufficiently from the cut ends to be reisolated. *Fusaria* will grow on most standard mycological media although when the primary need is to produce fructifications for identification purposes a weak medium is recommended. Gordon (1952) in Winnipeg found potato sucrose agar (1) eminently suitable for this purpose and this is the standard medium used at the

CMI. When the isolation plate has begun to sporulate it is strongly recommended that a series of single spore cultures be reisolated and these should be used for identification. If sporodochia are present it is often possible to isolate single spores from these without using an intermediate plate.

Isolation from Seeds

To detect the external mycoflora on pigeonpea seeds, fifty randomly selected seeds from each sample were divided into two groups, each was suspended in 10 ml. of sterilized distilled water in the conical flasks, separately. The flasks were shaken by hand for 10 minutes. After shaking, equal volume of this suspension was transferred into two centrifugal tubes. These tubes were kept in centrifuge and rotated at 2000-2500 rpm for 10 minutes. The supernatant liquid was decant-off from each tube and then the sediment from respective tubes was thoroughly mixed in 2.0 ml lactophenol Booth, (1971) and examined under compound microscope for the presence of fungal spores, fructifications and mycelial fragments.

The viability test of the fungal spores, fructifications and hyphal fragments present in the sediments was carried out by plating the water suspension of the sediment on 2.0 percent water agar in Petridishes. The dishes were incubated at $27 \pm 1^{\circ}C$ for 5 days and examined under stereoscopic binocular microscope for the presence of fungal colonies, if developed.

Four hundred seeds per sample in four replications, each of one hundred seeds were tested by Standard Blotter Method (ISTA, 1985). Ten randomly

selected seeds were plated on three layered moist blotters at equal distance with the help of sterilized forcep in each Petridish (9.0 cm diameter). These seeds were then incubated at a temperature of 27 ± 1°C under twelve hours alternating cycles of light and darkness for 7 days. The seeds were examined for the presence of mycoflora under stereoscopic binocular microscope.

In another experiment, four hundred seeds from each sample were pretreated by dipping the seeds, separately, in 1.0 percent solution of chlorine for a period of 10 minutes and examined by Standard Blotter Method as described above. At the end of incubation period, the fungal species growing on the seeds were transferred and cultured on Potato Dextrose Agar medium and purified by Single Spore Isolation and Hyphal Tip Isolation techniques. The pure cultures, thus obtained were stored on PDA in culture-tubes (i.e. slants) in the refrigerator at 8-10°C for further studies.

Four hundred seeds per sample in four replications, each of one hundred seeds were pretreated with 1.0 percent available chlorine as described in Standard Blotter Method and plated on PDA at the rate of 5 seeds per Petridish. The seeds were incubated at 27 ± 1°C for 7 days under twelve hours alternating cycles of light and darkness and examined macro-scopically by naked eyes for the presence of fungal colonies on seeds. Their specific identification was carried out with the help of standard identification manuals under stereoscopic binocular and compound microscopes.

The growth habit characters of each fungal species as it appeared and looked on pigeonpea seeds were recorded under stereoscopic binocular microscope (6.4 to 40 x magnifications). The cultural and morphological characters of each fungus were studied individually either on Malt Extract Agar or on Potato Dextrose Agar after 7-10 days of incubation. The morphological characters, i.e. shape, size and colour of the sexual and asexual structures of fungi were recorded and measured by the ocular and stage micrometer under compound microscope. The species of fungus were identified with the help of standard identification manuals or monographs.

Single *Fusarium* spores may be successfully isolated by standard dilution plate methods where the germinating spore is removed from the plate under a dissecting microscope by the use of a needle. Alternatively mechanical aids can be used, the simplest being the La Rue cutter or Keyworth's modified La Rue cutter. This replaces one of the objectives in the nosepiece of the compound microscope and should be parfocal with the rest. After a single spore has been selected by a suitable lens the cutter is swung round to cut out the spore.

To economise on the use of glassware involved in dilution techniques the spore suspension may be made as follows. A drop of sterile water is placed on a sterile slide under the dissecting microscope. An accumulation of spores is then obtained on the wet tip of a needle and the point of the needle introduced into the drop of water on the slide. The spores can then be observed as they leave the tip of the needle and flow into the water. When the suspension is adequate the needle

can be withdrawn. Experience of the correct dilution can easily be acquired and is approximately the point when the spores are clearly distinguishable in the water and are not obscured by overlapping. The spore suspension is then taken up in a loop and streaked across a plate.

Many isolations of *Fusarium* species have a tendency to produce abundant mycelium without forming spores and some stimulation is often required to initiate spore production. *Fusarium graminearum* is a notable example of this trend and Cappellini & Peterson (1965) found that they could stimulate conidial formation in this species by using a carboxymethylcellulose medium (7). They macerated a plate culture in 100 ml of sterile distilled water for 30 seconds in a Waring blender and 1 ml of this suspension was added to each test flask. The flasks were then shaken on a rotary shaker at approximately 250 rpm; abundant macroconidia were present after 4 days.

Joffe (1963) used a modified form of a medium Bilay's Medium modified by Joffe first proposed by Bilay (1955). This is a sugar-deficient medium and strips of pure cellulose lens paper were added. Joffe stated that this substrate always induces conidia in *Fusarium*.

Spore formation can also be stimulated by the use of a natural substratum. Grass, young leaves, bean stalks or pods, slices of carrot, turnip or potato have all been used. With care potato and slices of other root vegetables can be inoculated and used without sterilisation. Other material is first autoclaved and then stood in water or immersed in agar. For the examination of sporogenous cells autoclaved

blades of grass can be floated in water and inoculated. This reduces the mycelium and the conidiophores form more or less on the surface of the water. Hansen & Snyder (1947) found that autoclaved material did not greatly stimulate sporulation. They preferred to use propylene oxide to sterilise cereal straw which was then embedded in water agar. Macroconidial formation is stimulated and increased in most *Fusarium* cultures by light, and growth under a bank of two daylight fluorescent tubes mounted about 10-14 inches above the cultures is usually very effective. *Fusarium heterosporum* and other species causing head blights of cereals often respond to irradiation with near ultraviolet or black-light (Leach, 1962) and produce abundant sporulation usually in alternate bands corresponding to the 12 hr on/off cycle.

The light-benches used at the CMI have three 4 ft fluorescent lamp holders 6 inches apart. The centre holder has a Philips 'black-light' tube TL 40 W/08 and on either side of this is a daylight tube, Philips cool white MCFE 40 W/33. All three are controlled by one time switch set at a 12 hr on/off cycle. Fourteen inches below the fluorescent tubes is a shelf 48x20 inches to hold the Petri-dishes. These are standard 9 cm polystyrene disposable dishes which have approximately a 70% transmission of near-UV and a satisfactory screening effect of germicidal wavelengths. Dishes are streaked with non-sporulating isolates and grown in an incubator in the dark at 24°C for 2-4 days. They are then placed under the fluorescent lights until growth stops (5-10 days). It is often advantageous to seal the edge of the dish with transparent adhesive sealing tape to prevent too rapid drying of the culture medium.

To stimulate production of chlamydospores small squares of agar should be cut from a growing culture and placed in a sterile Petri dish in distilled water. Alexander et al. (1966) used sterile soil extracts obtained from poor sandy soil to induce chlamydospore formation. Qureshi & Page (1970) recommended the use of a solution of monobasic potassium phosphate and magnesium sulphate to which was added 0.125-2.0 mg of either glucose or magnesium carbonate. Chlamydospores formed abundantly after 3-4 days.

(1) Potato Sucrose Agar

Potato extract	500 ml
Sucrose	20 g
Agar	20 g
Distilled water	500 ml

The water and potato extract are mixed together and the sucrose and agar added. The mixture is heated slowly until the agar is dissolved and the pH adjusted if necessary to 6.5 with calcium carbonate. It is then dispensed in suitable bottles and autoclaved at 15 psi for 20 min.

(2) Potato Dextrose Agar

Potato (scrubbed and diced)	200 g
Dextrose	15 g
Agar	20 g
Distilled water	1000 ml

New potatoes should be avoided. Boil potatoes for 1 hr. and pass the mixture through a fine sieve, add agar and boil until dissolved, add dextrose and stir-autoclave at 15 psi for 20 min.

(3) Oatmeal Agar

Oatmeal (powdered)	30 g
Agar	20 g
Distilled water	1000 ml

Add oatmeal to water and gradually heat to boiling in a water bath or double saucepan and boil for 1 hr. Strain through muslin and make up liquor to 1 litre with water. Add agar and dissolved. Autoclave at 15 psi for 20 min.

(4) Tapwater Agar

Agar	15 g
Tapwater	1000 ml

Sterile wheat straw or rice grains may be added for use as a general medium. Many *Fusaria* spore well on this.

(5) Nash & Snyder's (1962) Peptone PCNB Medium

Difco peptone	15 g
Agar	20 g
Potassium dihydrogen phosphate (KH_2PO_4)	1 g
Magnesium Sulphate ($MgSO_4 . 7H_2O$)	0.5 g
Pentachloronitrobenzene (PCNB 75% wettable powder) 300 ppm streptomycin (when cooled)	1 g

(6) Papavizas' (1967) Peptone--PCNB modified.

Difco peptone	15 g
Agar	20 g
Potassium dihydrogen phosphate (KH_2PO_4)	1 g
Magnesium Sulphate ($MgSO_4 . 7H_2O$)	0.5 g
PCNB -- (Terraclor, a commercial product, is 75% active)	0.5 gm
Streptomycin Sulphate	100 mg

Chlortetracycline HCL	50 mg
Oxgall	0.5 gm

The last two components are thermolabile and should be added to the cooled agar.

(7) CMC medium for stimulation of sporulation in *Fusarium graminearum* (Cappellini & Peterson, 1965)

Carboxymethylcellulose (CMC 7MP - Hercules Powder Co.)	15 g
Ammonium nitrate (NH_4NO_3)	1 g
Potassium dihydrogen phosphate (KH_2PO_4)	1 g
Magnesium Sulphate ($MgSO_4 . 7H_2O$)	0.5 g
Yest extract	1 g
Distilled water	1000 ml

(8) Bilay's medium modified by Joffe

Potassium dihydrogen phosphate (KH_2PO_4)	1 g
Potassium Nitrate (KNO_3)	1 gm
Magnesium Sulphate ($MgSO_4 . 7H_2O$)	0.5 g
Potassium Chloride (KCl)	0.5 g
Starch Powder	0.2 g
Glucose	0.2 g
Sucrose	0.2 g
Distilled water	1000 ml

Strips of pure cellulose lens paper were added before the agar had set.

Media used to Increase Inoculum

(9) Cerelose Ammonium Nitrate Medium (Scheffer & Walker, 1953)

Cerelose	50 g
Ammonium Nitrate (HN_4NO_3)	10 g
Potassium dihydrogen phosphate (KH_2PO_4)	5 g
Magnesium Sulphate ($MgSO_4 . 7H_2O$)	2.5 g
Ferric Chloride ($FeCL_3 . 6H_2O$)	0.02 g
Distilled water	1000 ml

(10) Armstrong *Fusarium* medium

Sucrose or Glucose	20 g
Magnesium Sulphate ($MgSO_4 . 7H_2O$)	0.4 g
Potassium Chloride (KCl)	1.6 g
Potassium dihydrogen phosphate (KH_2PO_4)	1.1 g
Calcium Nitrate ($Ca(NO_3)_2$	5.9 g
Ferric Chloride ($FeCL_3$)	
Manganese Sulphate ($MnSO_4$)	0.2 ppm
Zinc Sulphate ($ZnSO_4$)	0.2 ppm

(11) Medium used to study staling of *Fusarium oxysporum* (Park, 1961).

Glucose	0.7 g
Magnesium Sulphate ($MgSO_4 . 7H_2O$)	0.5 g
Potassium dihydrogen phosphate (KH_2PO_4)	0.2 g
Ammonium Nitrate (NH_4NO_3)	0.1 gm
Agar	15 g
Distilled water	1000 ml

Wheat or rice straw or plant stems in Petri dishes or longer pieces standing in tap water agar or in water in large medical flats or other suitable containers.

Detection of Fusaric Acid

Fusarium species were grown for 20 days in 100 ml of Czapek's liquid medium in 500 ml Erlenmeyer flasks. The culture filtrates were collected and centrifuged at 2000 rpm for 20 min. The clear supernant was taken and the pH was adjusted to 4.0 by adding 2 NHCl. 100 ml of the filtrate was mixed with equal volumes of ethyl acetate atleast for 4 times in a separating funnel allowing 15 min for each extraction. All ethyl acetate extracts were mixed and evaporated to dryness. 1 to 2 ml of ethanol was added to dissolve the residue. Drops of known volume (0.005 ml) of filtrated as well as an index solation of fusaric acid were kept on Whatman's Filter Paper No. 1 Spotted chromatogram were run ascendingly for 10-12 hrs in butanol, formic acid and water (75 : 15 : 10). Chromatograms were dried and sprayed by bromophenol blue (0.04% in 90% ethyl alcohol). Fusaric acid gives a yellow colour.

ISOLATION AND PATHOLOGICAL STUDIES

As the identification of *Fusarium* species is based on their cultural characteristics and their full range of spore morphology it is necessary to maintain cultures for comparative identification. Also many heterothallic species rarely produce perithecia in nature as under field conditions vegetative propagation appears to predominate and one strain only may occur in a given area. When opposite mating types have been obtained they are valuable cultures and should be maintained. A further aspect is the maintenance of pathogenic strains for pathogenicity testing on different hosts, some of which may be seasonal or slow growing. Suitable culture maintenance is also required for research into laboratory methods of determining pathogenicity or toxicity. Maintenance of cultures on agar, even if growth is slowed down by covering the surface with mineral oil, is largely unsatisfactory. *Fusarium* cultures are for the most part extremely lable. As long ago as 1926, Brown said the capacity of *Fusarium* species to mutate was a function of the culture media. He noted that on rich synthetic media such as Richards' it was difficult to maintain the parent strain for more than two generations. Rich media certainly stimulate mutations or cultural adaptations which may appear as a change of form of the morphology, cultural characteristics and even the biochemical factors. These changes take place due to the adaptation of the fungus to an artificial environment whether on rich or weak media or even when growth is slowed down to a minimum by refrigeration or by covering the

surface of the culture with mineral oil. We have therefore to look to methods of preservation which induce dormancy such as freeze-drying (lyophilisation) and storage in or above liquid nitrogen. A simple and less expensive method for those with limited facilities is storage in dry soil. This is not entirely satisfactory because of the time lag before the onset of dormancy and also because a few species do not appear to survive by this method.

Miller (1945a) and Cormack (1951) described the successful maintenance of *Fusarium* species in sterile loam soil. Cormack (1951) found that *Fusarium avenaceum* survived three years in soil at room temperature and up to eight years if the soil is kept under refrigeration at about 5°C. The method consists of placing a few grams of sieved sandy loam soil, not clay, in a container, preferably a durable one such as a McCartney bottle, which is one third filled with soil. The bottle and soil are then autoclaved twice at 20 psi for 30 min at an interval of 3-5 days. The tops with their rubber liners are then screwed down until required. To preserve a culture a spore suspension is made from the parent and about 2 ml of this is poured over the soil. This is then allowed to grow for several days with the cap loose. After this time has elapsed the cap is tightened and the culture stored preferably in a refrigerator at 4°C. When a fresh culture is required a few particles of soil are removed from the bottle and sprinkled over the surface of an agar plate. As the *Fusarium* grows out from the soil particles it is immediately subcultured so as to remove it from any particles that may interfere with future examination.

A comprehensive survey of various field of Allahabad, Azamgarh, Ambedker Nagar, Mau, Ballia, Ghazipur and its adjacent regions were made and various wilted crop plants, trees, soil and seed samples were collected. Blotter Agar Plate and Blotter Roll methods, were used for isolation of Fusarium species. Fusaria were isolated, purified and maintained on Malt-extract and Potato Dextrose Agar media. Morphological studies were carried out and identifications were made.

Fusaria isolated from soil, seed samples and wilted trees at various places are as follows :

Table : 1
Fusaria isolated from various crops field, wilted trees and seeds

***Fusaria* isolated from soil**	***Fusaria* isolated from seeds**	***Fusaria* isolated from wilt trees**
Allahabad District		
Fusarium oxysporum	*Fusarium. semitectum*	*Fusarium oxysporum*
F. solani	*F. acuminatum*	*F. equiseti*
F. equiseti	*F. culmorum* *	*F. lateritium*
F. acuminatum	*F. heterosporum*	*F. nivale*
F. roseum	*F. nivale*	*F. decemcellulare* *
Azamgarh District		
F. poae *	*F. solani*	*F. moniliformae*
F. oxysporum	*F. semitectum*	*F. sambucinum*
F. graminearum	*F. roseum*	*F. sulphureum* *
F. solani	*F. oxysporum*	*F. equiseti*
F. equiseti	*F. sambucinum* *	*F. oxysporum*
F. xylarioides *	*F. acuminatum*	*F. heterosporum*
F. stilboides *	*F. fusiroides*	*F. avenaceum*

Ambedker Nagar District

F. acuminatum	*F. nivale*	*F. decentaelluelare* *
F. oxysporum	*F. sumbacin* *	*F. oxysporum*
F. culmorum	*F. graminearum*	*F. roseum*
F. lateritium	*F. xylarioides*	*F. acuminatum*
F. avenaecum	*F. heterosporum*	*Fusarium sp.*
F. moniliformae	*F. oxysporum*	*Fusarium sp.*
F. solani	*F. equiseti*	*F. semitectum*

Mau District

F. oxysporum	*F. stilboides* *	*F. heterosporum*
F. equiseti	*F. literitium*	*F. moniliformae*
F. solani	*F. culmorum*	*F. oxysporum*
F. roseum	*F. decemcellulare* *	*F. acuminatum*
F. heterosporum	*F. avenaceum*	*F. solani*
F. acuminatum	*F. oxysporum*	*F. graminearum*

Ballia District

Fusarium acuminatum	*F. equiseti*	*F. sambucinum* *
F. solani	*F. juruanum* *	*F. lateritium*
F. tabacinum *	*F. arthrosporiodes*	*F. nivale* *
F. stoveri	*F. moniliformae*	*F. oxysporum*
F. buxicola *	*F. larvarum* *	*F. illudens* *
F. gigas *	*F. poae*	*F. sphaeral* *
F. concolor *	*F. epistromum* *	*F. fusaioides*
F. arthrosporioides	*F. oxysporum*	*F. semitectum*
	F. solani	

Ghazipur District

F. oxysporum	*F. heterosporum* *	*F. oxysporum*
F. equiseti	*F. gigas* *	*F. stoveri* *
F. roseum	*F. fusiroides*	*F. stilboides* *
F. poae *	*F. solani*	*F. moniliformae*
F. acuminatum	*F. acuminatum*	*F. arthrosporioides*
F. solani	*F. concolar* *	*F. acuminatum*
F. graminearum	*F. xylarioides* *	*F. lateritium* *
		F. equiseti

* Fusarium species were reported from the first five in related areas :

Isolation studies revealed that out of 36 species of Fusarium, 20 species from soil samples, 26 species from seed samples and 23 species from wilted trees were isolated.

STUDIES ON PATHOGENICITY

The pathogen is an agency which generates suffering in plants and the pathogenesis is the process for the appearance of particular disease and includes the action of pathogen. The susceptibility of the host and the impact of the acillary factors. That quality or the ability of pathogen of generate carries no implication of how desease is caused to prove that a certain organism establish the Roch's postulates, i.e., isolation of suspected pathogen from the infected plant tissue in order to establish the identity in culture. The culture is inoculated on other healthy plants develop the same type of disease symptoms and reisolation of organism from the artificially inoculated plants with identical characters.

The disease starts with yellowing and drying of upper (terminal) leaves in the outer shoots of a tree. Symptoms start to appear after 15-30 days of rains. The drying of leaves progresses downwards and very rapidly upto the junction of the branch. Sometimes all the leaves of a single branch show epinasty the petioles bend downwards, forming and obtuse angle with the main stem. The wilted leaves become dry and brittle.

The unilateral wilt symptoms were also observed in various cases which clearly differentiate this pathological wilting phenomenon from physiological wilting. This may be due to the infection of roots and stems on one side only and the consequent confinement of the pathogen to that side only (due to restricted

lateral movement of the pathogen). The common observation was that disease do not develop uniformly, for example, if a branch has two shoots which are on opposite directions, one may show wilting and drying of the leaves and other may have healthy green leaves.

The general health of the tree also deteriorates and some other important symptoms like poor growth of the young shoots and chlorosis of the older leaves were also observed. In general it took 3-4 months for a healthy tree of all the fruit crops under investigation (30 years old) converting into a dried specimen with few mummified guava fruits still attached to the dried branches along with some wilted (dried) leaves. The same species of *Fusarium* viz., *F. solani, F. oxysporum, F. equiseti, F. semitectum* and *F. moniliforme* soils of different orchards around Allahabad were also taken for pathological studies. These studies were carried out from the same stock cultures and hence the methods used for isolating, subculturing etc., were similar to those described earliar. For pathogenicity tests Arhar, Pea in Seed, Mango and Guava seedlings of same size and age (6 month old) were taken. Pathogenicity tests were carried out by the following methods.

1. The seedlings root both uninjured and injured (10 injuries by sterilized needle per root) were dipped in spore suspension (about 100 spores per lower field of compound microscope) of *Fusarium* species. The seedling were replanted in plastic pots. Controls were simultaneously maintained.

2. The seedlings were kept in culture tubes containing 20 days old culture filtrates of *Fusarium* species. In case of control, seedlings were kept in sterilized distilled water. Daily observation were made.

Reisolations were always made in order to confirm the infection with particular *Fusarium* species. Ten seedlings per treatment were taken in each case. The results of both set of experiments are summarized in the table.

Results from the pathogenicity test (first set) clearly show that all the five species of *Fusarium* were capable of causing wilt of Arhar, Linseed, Pea, Mango and Guava seedlings. The wilting was characterised by gradual withering, yellowing and drying of leaves. Later on it was followed by drying of entire seedlings. It was observed that there was slightly higher percentage of infection in case of injured root seedlings than those uninjured. Out of the five species *F. oxysporum* was more pathogenic to Arhar, Linseed, Pea, Mango and Guava seedlings than the other two species, as it caused a higher percentage of wilting in the seedlings.

Results from another set, where the seedlings were kept in culture filtrates of *Fusarium* species, showed that seedlings wilted within seven days. All the seedlings kept in culture filtrates of *F. oxysporum*, *F. solani* and *F. equiseti* wilted while in *F. semitectum* and *F. moniliforme* the percentage was a little less. Controls remained healthy in both set of experiments. Thus from the above

experiments it is clearly evident, that *F. oxysporum, F. solani* and *F. equiseti* causes more damage than other one species of *Fusarium* and from second set that some toxic substance also plays a role in causing wilting of Arhar, Linseed, Pea, Mango and Guava seedlings. An attempt was therefore made to detect the toxic substance (Fusaric acid) in the culture filtrates of the five species of *Fusarium.*

Aqueous mycelial and spore suspensions of the organisms prepared from 7 to 10 days old cultures, were sprayed on injured as well as uninjured leaves and stems of their suspective host plants with the help of hand automizer. Humidity was maintained by covering it with polythene bag with some sterilized water at the base. Suitable controls were maintained for each treatment. In each case similar conditions such as amount of inoculum, water and same kind of soil etc., were provided. Fungi which were found to cause root-rot stem-rot wilt and seedling bli-lights and leaf-spots.

Table : 2

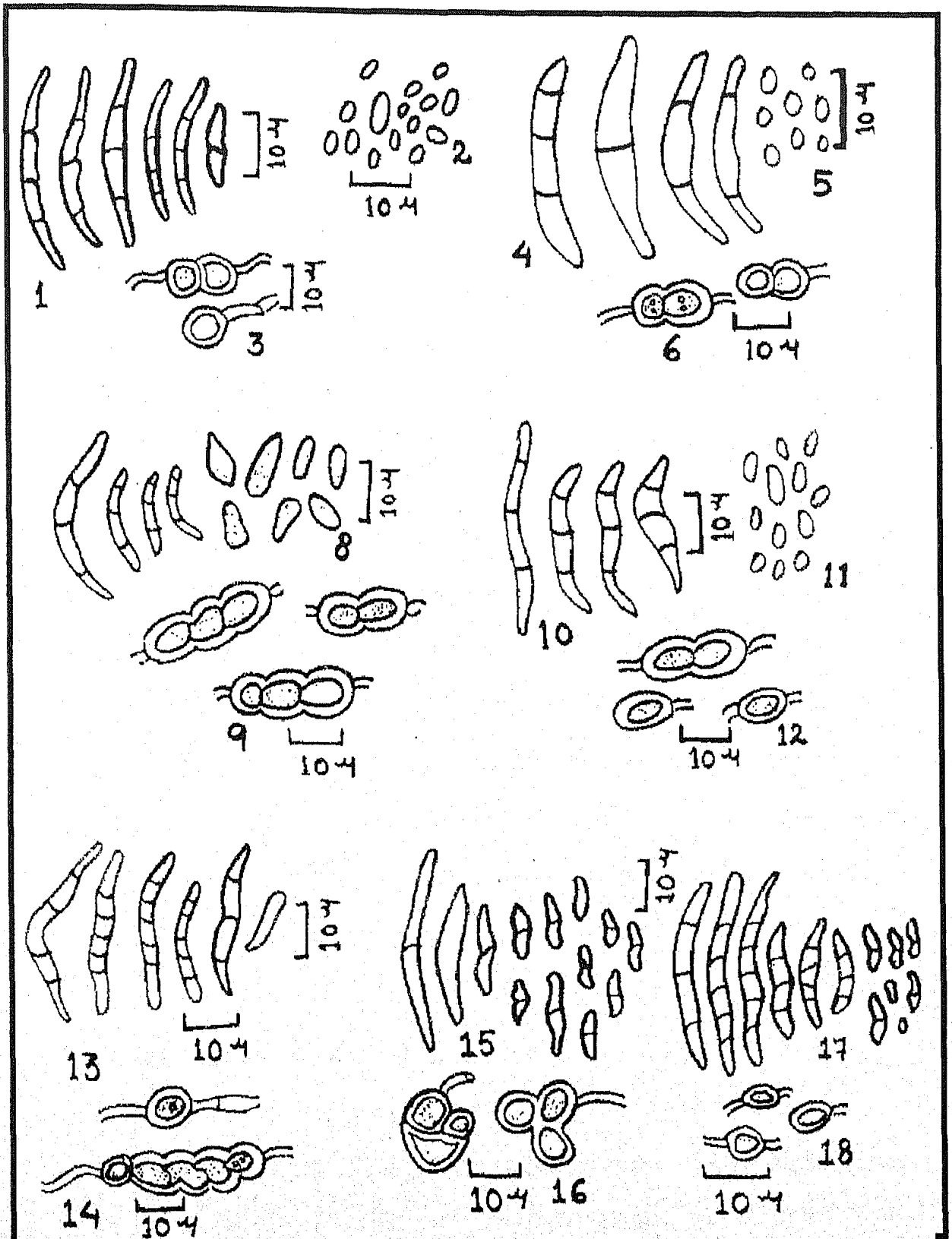
Pathogenicity test and percentage wilting of various crops seedlings

Crops	Organism	Percentage of wilting		
		After 1 Week		After 4 days in culture filtrate
		Uninjured	Injured	
Arhar	*Fusarium solani*	40	50	70
	F. oxysporum	55	65	100
	F. equiseti	50	55	65
	F. semitectum	20	25	50
	F. moniliformae	35	45	55
	Control	Nil	Nil	Nil
Lin-seed	*Fusarium solani*	50	60	70
	F. oxysporum	55	65	80
	F. equiseti	40	50	55
	F. semitectum	20	30	50
	F. moniliformae	30	40	45
	Control	Nil	Nil	Nil
Mango	*Fusarium solani*	45	60	65
	F. oxysporum	50	65	70
	F. equiseti	50	60	70
	F. semitectum	40	55	60
	F. moniliformae	40	50	65
	Control	Nil	Nil	Nil
Guava	*Fusarium solani*	54	60	75
	F. oxysporum	62	74	100
	F. equiseti	50	60	75
	F. semitectum	48	55	65
	F. moniliformae	26	36	65
	Control	Nil	Nil	Nil

Table : 3

Showing Pathogenicity Results

Crops	Pathogen	Disease	Symptoms
Arhar (*Cajanus cajan*)	*Fusarium oxysporum*	Wilt and seedling rot	The leaves of affected plants turn yellow pre-maturely, the foliage droops and within three or four days the plant dry up. The first symptoms of the disease is yellowing of the leaves. Within a day or two such leaves drop and in the course of next two or three days they may drop off, when the stem is examined closely, dark lesions may be seen on the bark at the ground level.
Linseed (*Linumusititasinum*)	*F. solani*	Wilt and root-rot	Plant show stunting dropping and chlorosis of leaves. Stem also shows shrinkage in lower portion roots are poorly developed
Pea (*Pisum sativum*)	*F. equiseti*	Root-rot	Root start gradual, roots poorly develop and finer root lets get destroyed.
Mango (*Mangifera indica*)	*F. acuminatum*	Wilt and Root-rot	Lack of root hairs and other secondary roots, yellowing and shrivelling of leaves and finally wilting.
Guava (*Psidium guajava*)	*F. semitectum*	Wilt	Lack of root hairs and other.


1
2
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15
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17
18
10μ

MORPHOLOGICAL STUDIES OF *FUSARIUM*

Spore morphology is the major character in the identification of *Fusaria.* Spores may be conidia produced as simple or polyphialidic slime spores or as enteroblastic spores (i.e. a conidium produced through a pore without the involvement of the outer wall of the conidiophore and enlarging without the formation of a septum), or chlamydospores which in many species form the resting stage. Conidia may occur as 0-1 septate, pyriform, fusoid to oval microconidia through to straight or curved, 0-10 or more septate macroconidia.

The presence or absence of microconidia and chlamydospores together with the shape of both the micro-and macroconidia were used by Wollenweber (1913-35) to establish sections or groups within the genus. The species placed within the respective sections were to some extent phylogenetically related, and further evidence from other aspects such as pigmentation and the discovery of the perfect states of many of the species has confirmed the overall relationship within the section. All the perithecia belong to the Hypocreales and are placed in the following genera : *Nectria (Hypomyces* is incorrectly used for these species), *Calonectria, Gibberella* and also *Micronectriella* which is now used in this paper for the perithecia of the Arachnites section. These genera are all closely related; *Nectria* has 1-septate ascospores and *Calonectria* more than one. *Gibberella* differs from the latter in having a blue pigment in the cells of the perithecial wall

and *Micronectriella* has immersed perithecia on the host. Matuo & Kobayashi (1960) described *Fusarium splendens* as the conidial state of *Hypocrea splendens* Phil. & Plowr. but this has not been repeated or material seen.

For many years the sporogenous cell in *Fusarium* has been regarded as a phialide Mason, (1933) and micro-and macroconidial forms have long been recognised for many species. Available evidence suggests that probably all *Fusarium* species have developed from forms with two conidial states. *F. culmorum* which does not normally possess a microconidial state will form one if the macroconidia are induced to germinate under adverse conditions. The presence of blastospores in the section Arthrosporiella may represent the survival of a primitive character or a secondary return to two conidial forms. The dry spore nature of the blastospores certainly helps air dispersal. These blastospores may form before or simultaneously with the normal phialospores; they occur as the microconidial form in *F. sporotrichioides* and *F. fusarioides* and as macroconidia in *F. semitectum* and *F. avenaceum.*

The morphology of the macroconidia as used by Wollenweber and other workers for the establishment of the various sections has been observed. Because the sporogenous cells are regarded as of more fundamental taxonomic importance than macroconidial shape, the sections Arthrosporiella and Gibbosum have been modified and the section Roseum discarded. The Martiella section has thick-walled, curved, dorsiventral conidia in which a large percentage of the spores have

the widest diameter in the upper half of the spore. The Elegans and Liseola sections have thin-walled indistinctly septate macroconidia, evenly curved fusoid and with the widest part in the middle. Lateritium has cylindrical macroconidia curved towards the apex and base and often with a beaked apical cell whereas the section Gibbosum has strongly dorsiventral macroconidia often with a pedicellate basal cell and with the apical cell elongated to form an acicular tip.

When microconidia are formed in chains these are most easily observed by placing an opened Petri-dish under the low-power lens of a compound microscope. There is no difficulty in separating *F. moniliforme* from *F. decemcellulare,* the only two species which form their microconidia in chains, but the presence of conidial chains is a useful simple method for the early separation of *F. moniliforme* from *F. oxysporum.* Blastosporic species are also easy to recognise as dry conidia under the low power of the compound microscope and the cultures have a powdery appearance.

Many species of *Fusarium* produce stromatic pustules which may be perithecial initials or develop into true sporodochia. The term derives from Greek words spora-spore and docheion-holder (latinised to dochium). The sporodochium is a fructification in which the conidial mass is supported by a cushion-like (pulvinate) aggregation of short conidiophores.

The slow growing species especially in the Episphaeria and Arachnites sections have pionnote sporodochia. Pionnotes comes from Fries (1849) who used the term for some *Fusarium* species which produce a mass of spores having a fatty or greasy appearance. Sherbak off (1915) stated that some species of *Fusarium* produce minute sporodochia close to the surface of the substratum without any stromata. When these sporodochia produce spores they form a continuous slimy layer. He redefined this type of fructification as pseudopionnotes although a better term is pionnote sporodochia.

Pigments are not merely by-products but as noted by Mull & Nord (1944) they are involved in the enzymatic activities of the organism. Pigments were isolated as early as 1904 (Bessey) and between 1934 and 1940 much work on *Fusarium* pigments was done at the London School of Hygiene and Tropical Medicine by Professor Raistrick and his associated workers. Their extraction and analysis of the pigments of *F. culmorum* was particularly noteworthy; they separated three pigments which they called rubrofusarin $G_{15}H_{12}O_5$, aurofusarin $G_{30}H_{20}O_{12}$ and culmorin $G_{15}H_{26}O_2$. Other pigments such as javanicin, bostrycoidin, solanione and lycopersin have since been isolated from *F. solani* and *F. oxysporum*; these latter are generally regarded as being naphthoquinones.

Benada (1963) found by chromatographic analysis that the mycelial pigment in *F. nivale* consists of orange, red, yellow and two carmine components.

Its presence provides a rapid means of separation from other cereal fusaria such as *F. culmorum, F. avenaceum* and *F. graminearum.*

***Fusarium semitectum* Berk. & Rav.**

Whitish or light orange white, compact mycelial growth covering the whole seed was seen alongwith the characteristic whitish, shiny, highly branched conidiophores bearing easily recognizable macroconidia giving an appearance of 'flower'. Branched conidiophores with macroconidia easily be observed on the periphery of colonies.

Culture - Colonies on PDA whitish incarnate or isaballin with abundant aerial mycelium of whitish cottony growth in the early stages and later changed into light yellow or buff brown; Mycelium septate, 3.0-6.0 μ thick, brand creeping; Conidiophores arise singly or in groups, whitish, shiny, highly branched bearing macroconidia; Macroconidia developed on aerial mycelium as fusoid, hyaline, 0 to 5 septate but occasionally upto 7 septate, wedgeshaped, measured on the basis of septation.

No. of septa	**Size of macroconidia**
0	4.0-16.0 x 2.0-4.0 μ
1	8.0-20.0 x 2.0-4.5 μ
3	23.0-50.0 x 3.2-6.2 μ
5	32.0-65.0 x 4.0-6.5 μ
7	37.0-77.0 x 4.2-6.5 μ

Microconidia not observed.

Chlamydospores not observed.

(2) *F. acuminatum* Ellis and Everh.

Culture On PDA the mycelium varies from white to brownish red, and frequently but not always produce a deep red or carmine red colour in contact with agar. Sporodochia are red to salmon coloured.

Microconidia Develope in aerial mycelium from simple phialides, measuring 12 - 16x3 - 4 μ in size.

Macroconidia Broadly falcate, strongly dorsiventral 3-5 septate, measuring 30-55 x 4.0-4.8 μ in size.

Chlamydospores Intercalary, thick walled globose to oval measuring 10-15 x 15-18 μ.

Booth (1971) has, however described this species with 3-7 septate macroconidia.

(3) *Fusarium oxysporum* Schlecht.

Culture On PDA the fungus produces a violet pigment in contact with agar. *Sclerotia* are usually blue-black. Sporodochia are cream to salmon coloured.

Microconidia Borne on simple phialides, generally abundant, variable, ellipaoid, cylindrical straight to curved, 5-12 x 2.2-3.5 μ in size.

Macroconidia Thin walled, generally 3-4 septate, fusoid-subulate and pointed at both ends, 25-45 x 3.0-4.5 μ in size.

Chlamydospores Smooth walled, abundant, both terminal and intercalary, generally solitary but occasionally formed in pairs or in chains, they are 5.5-8.0 x 5-7 μ in size.

(4) ***Fusarium solani*** **(Mart.) Sacc.**

Culture On PDA the cultures show many colour variations, the most common being to greyish-white. The pigment may or may not be produced in the agar. If develops usually it is blue to bluish-brown.

Microconidia Develop abundantly, oval in shape with somewhat thicker walls and measure 8-16 x 2-4 μ.

Macroconidia Inequilaterally fusoid, generally 3-7 septate. The apical cell is pointed and somewhat beaked, measuring 35-55 x 4.5-6 μ.

Chlamydospores They are globose to oval, smooth walled and are formed terminally on short lateral branches or are intercalary, measuring 9-12 x 8-10 μ.

(5) ***Fusarium semitectum***

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The characteristic symptoms of disease in the field condition were, browning of the leaves, and wilting of the plants. When uprooted, the plants easily came out from the soil due to their decay of roots system by the attack of

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pathogen. Brownish lession were found on the collar region of the plants. The infected portion after proper sterilization, when isolated on Potato Dextrose Agar, the Fungus *Fusarium moniliforme* Sheld. was obtained.

When the fungus was tested for its pathogenicity in pot culture experiments, the first symptom of the disease occured 15 days after sowing, when the plant was 6" to 9" in height, starting from yellowing of leaves and ultimately the plants wilted. The roots shreaded out due to decaying. Dark brown lessions were observed on the sheath or collar region of the plants.

The fungus reisolated from the affected portion of the plant and examined under microscope. The organism was cultured on Potato Dextrose Agar. The characteristic of the fungus were as follows :

Mycelium	:	Septate, hyaline, branched.
Conidiophore	:	Simple or branched, usually apposite, hyaline, bearing conidia in chains or in clusters.
Conidia	:	Conidia are of two types.
Micro conidia	:	Continuous, oblong, avoid, moniliforme, 4 to 10 μ and 2 to 3.5 μ in length and breadh respectively. hyaline.
Macro conidia	:	Hyaline, curved, especially, near the apex and acute at the tips. 3 to 5 septate, measuring 2.9 μ to 3.2 μ by 25 μ to 40 μ and 3.0 μ to 3.2 μ by 40 to 55 μ in breadth and length respectively.

DISEASES CAUSED BY PATHOGENIC *FUSARIA*

Typically the wilt diseases are associated with plants that contain vessels in their xylem, that is, the angiosperms. While wilt, or loss of turgidity, is a common symptom of disease, it may result from one of many possible causes. The vascular wilts are set off as a group because of the association of symptoms with the presence of the pathogen in the vascular system of the host, usually in the xylem tissue. The most common pathogens are vascular *fusaria* life cycle of a typical vascular pathogen is depicted in *Verticillium* sp. and bacteria for example, the pathogens of cucurbit, bacterial wilt and southern bacterial wilt. The wilt syndrome includes vein clearing, epinasty, chlorosis, vascular discolouration, stunting, temporary and permanent loss of turgidity and death. Apart from wilting, the syndrome of this group of diseases is not unique. The browning reaction occurs both in local lesion and wilt diseases as a result of similar sequences of biochemical reactions. Respiration is accelerated and auxins, phenols and other products accumulate in a similar manner in the two cases.

Water relations are drastically changed in infected plants. The water content in diseased plants diminishes and eventually dries up altogether. Stress initially

occurs in the issue of individual leaves and then throughout the plant. The water in the leaf blades is affected before mycelium enters them. In infected plants, leaves lose turgor and eventually die and become dissicated. The mechanism by which this occurs is debatable. The debate centres around two theories put forward to explain the phenomenon of wilting. The first theory is called the "toxin theory" and the second the "plugging theory" or "vascular occlusion theory". The toxin theory is based on the fact that the permeability of parenchymatous cells in leaves increases so that they lose their osmotic efficiency and this results in wilting, Gaumann (1951, 1954, 1957).

When there is very high resistance to the flow of water through the xylem, the leaves receive less water than they transpire and as a consequence, they wilt. This is the basis of the plugging theory, Ludwig (1952).

Table : 4

Diseases produced by the pathogenic *Fusaria*

Pathogen	Host	Common name
Fusarium oxysporum		
f. sp. lycopersici	*Lycopersicon esculentum*	Tomato wilt
f. sp. conglutinans	*Brassica oleracea*	Cabbage yellows
f. sp. redolens	*Numerous*	Vascular wilts
f. sp. vasinfectum	*Gossypium hirsutum*	Cotton wilt
f. sp. tracheiphilum	*Glycine max, and Vigna sinensis*	Soybean & Cowpea wilt
f. sp. niveum	*Citrillus lanatus*	Watermelon wilt
f. sp. melonis	*Cucumis melo*	Cantaloupe wilt
f. sp. batatas	*Ipomoea batatas*	Sweet potato wilt
f. sp. cucumerinum	*Cucumis sativus*	Cucumber wilt
f. sp. elaeidis	*Elaeis guineensis*	Oil-palm wilt
f. sp. albedinis	*Phoenix dactylifera*	Date palm Bayoud disease
f. sp. lini	*Linum usitatissimum*	Flax wilt
f. sp. cumini	*Cuminum cyminum*	Cumin wilt
f. sp. udum	*Cajanus cajan*	Pigeon pea wilt
f. sp. pisi	*Pisum sativum*	Pea wilt
f. sp. phaseoli	*Phaseolus vulgaris*	Bean root-rot
f. sp. ciceri	*Cicer arietinum*	Wilt
f. sp. lentis	*Lens Esculehta*	Wilt of lentil
f. sp. psidii	*Psidium guajava*	Wilt
F. solani	*Psidium guajava L.*	Wilt
F. moniliformae	*Mangifera indica*	Mango Malformation
F. moniliformae sheldon & f. sacchari	*Saccharum affecinerum*	Wilt

Wilt of Pigeon pea (*Cajanus cajan* L.)

This is a very common disease. It is the worst disease of red gram in India, causing severe damage wherever the crop is grown, especially in Maharashtra, Madhya Pradesh, Uttar Pradesh, Bihar and Tamil nadu.

Symptoms

The arhar plant is susceptible to the attack of wilt throughout its development. However, the symptoms are more pronounced and the damage greater when the plants develop after the rainy season. The main symptom of the disease is the wilting of seedlings and adult plants as if from water shortage even when plenty of it may be present in the soil. The wilting is characterized by gradual, sometimes sudden, yellowing, withering and drying of leaves followed by the drying of the entire plant or some of its branches. The main roots and base of the stem tissues are blackened either uniformly, or especially in the early stages, in streaks. If the stem is examined after it is broken and split, the black streaks may be traced up to a height of several feet on the stem. Partial wilting is also common, that is, often only one side of the stem and root system is affected in which case the symptoms are found only on that side of the plant.

Wilt of Linseed (*Linum usitatissimum* L.)

The wilt disease of flax or linseed plant is very widespread and occurs in almost all the countries where linseed is grown.

Symptoms

The plants are attacked during all stages of their growth. The tops of the plants droop and wilt, after which they die. The plants become yellowish before they wilt. The roots of young seedlings may rot and the plants may drop off. The mature plants, if infected, remain stunted.

Wilt of cotton (*Gossypium hirusutum)*

Wilt is one of the major disease of cotton and is found wherever this crop is grown. It is believed to have originated in Mexico or Central America, spreading to many African countries, parts of France, Italy, Yugoslavia, USSR, India and the West Indies. In India, the disease was first reported in Nagpur in 1908. The disease has now spread to almost all the black cotton soil tracts of the country. The pathogen causes extensive damage in the heavy soils and is more or less absent in the loamy soils of the northern plains and the Indo Gangetic plains.

Symptoms

The plants are affected during all stages of plant growth. The first symptoms appear on young seedlings. These are veinclearing of the leaves followed by interveinal tissue necrosis. There is yellowing and browning of the cotyledons and the formation of a brown ring on the petiole. The seedlings soon wilt and die. If the plants are affected a little later, the leaves become flaccid which results in dropping and wilting, starting from the oldest leaves at the base

and proceeding upwards, finally involving the branches and the whole plant. Often there may be complete defoliation, leaving the stem alone standing in the field. Discolouration of the stem may also be seen. The basal portion of the stem in young plants may turn black. In adult plants, the discolouration may be partial. Growth is retarded and the plant eventually wilts.

Panama disease of Banana

The disease is widespread throughout Africa, Asia, Australasia and Oceania, West Indies and North, Central and South Africa.

Symptoms

Reactions to the two clonal groups of cultivars differ to this disease.

1. 'Inodoratum' or yellowing strain which is characterized by a strong yellowing of erect leaves; this type is not found in Costa Rica, Panama, Columbia and Equador.

2. 'Odoratum' which is found in the Carribean Islands, Honduras and Gautemala. Plants of this type, infected by Panama disease, do not yellow but suffer from the collapse of the petiole. The cut stem smells badly of rotten fish.

In Gros Michel, a very susceptible variety, the lower leaves become yellow and the petioles collapse around the pseudostem from the base to the top. If the base of the plant is cut open vertically, numerous brown and black lines can be

seen running in all directions through the corm and upwards into the leaf bases and petioles.

The time between the rootlet infection and wilt is about two months. As a result of infection, the plant produces many suckers which are invariably infected with the fungus.

Fusarium diseases of Beans, Peas and Lentils

Beans (*Phaseolus vulgaris*), peas (*Pisum sativum*), and Lentils (*Lens esculenta*) are among the most important and widely grown food crops in the Leguminosae. These crops have been cultivated since ancient times and are important sources of protein. *Fusarium* diseases are serious factors limiting production and are prevalent wherever each crop is grown throughout the world.

Bean Root Rot

Fusarium root rot of bean has been studied widely, for its own sake and as a model for establishing microbiological principles relating to many soil-borne fungus diseases. The disease was first described by Burkholder in western New York State in 1916 Burkholder (1916), Burkholder (1919) where as many as 90% of the plants in several countries were infected. This diseases, often called "dry root rot of bean", has been considered a serious disease in most bean-producing areas of the world. Initial symptoms are reddish streaks on the hypocotyl and tap root which are evident about one week after plant emergence. This reddish

discoloration increases and coalesces to eventually cover the entire below-ground stem and root system, giving them a brown, corky appearance.

Roots of bean plants which are widely spaced and in soil conducive to good growth become large and vigorous in spite of the presence of *Fusarium* Burke (1965). In conditions unfavourable for optimum root growth, *Fusarium* reduces root volume and efficiency. The primary roots may be killed and adventitious roots arising from the hypocotyl just below the soil surface will keep the plant alive and productive, if soil moisture is adequate.

Crop losses from Fusarium root rot of beans can be severe. The disease in central Washington Burke *et al.*, ((1967) reduced seed yields among 12 cultivars from 6% (181 kg/ha) in relatively resistant Sutter Pink to 43% (2141 kg/ha) in highly susceptible Red Mexican UI-36, where productivity was measured in adjacent noninfested and *Fusarium*-infested fields. In New York Natti *et al.*, (1971) yields were increased 25-50% by chemical controls. In Colorado Keenan *et al.*, (1974) yield from dryland pinto beans was reduced as much as 84% in 1971. Estimated losses of 11-26% were attributed to root rot in Nebraska Steadman *et al.*, (1975).

Pea Wilt (*Pisum sativum*)

Fusarium wilt of peas, caused by *Fusarium oxysporum* f. sp. *pisi* race 1, was first reported in Wisconsin in 1924 Jones *et al.*, (1925). Reports in 1928 and

1932 established that this disease was prevalent in all growing areas of the United States and was one of the most important diseases affecting processing, dry and seed pea production fields Kadow *et al.*, (1932). Resistance to this disease was quickly found and was attributed to a single dominant gene. The introduction of wilt-resistant cultivars resulted in complete control of this disease in the commercial pea-growing industry. By 1942, a pea disease survey in Wisconsin failed to reveal race 1 of the wilt *Fusarium* in any commercial field. Furthermore, race 1 was not found to be an economic problem in the United States again until 1972, when the disease appeared in eastern Washington in a field planted to a susceptible cultivar. Race 1 has not been eliminated, but the disease is under control through the use of resistant cultivars.

Fusarium oxysporum f. sp. *pisi* race 2 was described in 1933 and has been reported from most pea-growing areas of the world Snyder (1933). Like race 1, resistance to race 2 was quickly found and resistance was again attributed to a single dominant gene Hare *et al.*, (1949). However, economic losses due to this disease have not been sufficient in the eyes of the pea industry to mandate the growing of resistant cultivars.

In 1963, a *Fusarium* wilt was observed in one field in Skagit County, Washington and in 1967 it was determined that this was a new race of *Fusarium* wilt Haglund (1968). This disease, which by 1969 had spread to more than 240 fields encompassing 4047 ha, was designated *F. oxysporum* f. sp. *pisi* race 5 in

1970. Yet another race of pea wilt *Fusarium*, capable of wilting pea cultivars resistant to races 1, 2 or 5 was also detected in 1970. This wilt, caused by a *F. oxysporum* tentatively designated race 6 (new race), has been detected in approximately 100 fields in western Washington, from the Canadian border to the Montesano area in southwestern Washington. The occurrence of the new strain was simultaneously observed in four locations in western Washington, geographically separated to such an extent as to preclude its having been originally disseminated to all these locations by wind, water, man's movement of equipment, vines, or contaminated soil. Seed contamination is considered a possible means of the original spread.

In 1974, *F. oxysporum* f. sp. *pisi* was reclassified into 11 races, including previously described races 1, 2 and 5 Armstrong *et al.*, (1974). Later, Kraft and Haglund obtained isolates of these reclassified races from the American Type Culture Collection. Rockville, Maryland, for study. All 11 isolates were determined to be either race 1 or 2 on the basis of standard inoculation procedures with 8 differential cultivars of known genetic resistances to races 1, 2 and 5. Hubbeling N., (1974) found that races previously described as 3 and 4 Boltan *et al.*, (1966), are actually both race 2. In view of this evidence, it appears that isolates previously designated as races 3, 4 and 6 to 11 of *F. oxysporum* f. sp. *pisi*, in reality, are race 1 and 2 types that vary only in virulence and not specific pathogenicity. In the evaluation of pathogenic forms of *F. oxysporum* f. sp. *pisi* it

is essential to use differential cultivars of pea with known genes for resistance together with a standard inoculation procedure, if consistent and reproducible results are to be obtained Haglund (1974).

In northwest Washington and southwest British Columbia (Canada), where peas have been growing essentially in monoculture for many years in the presence of races 1 and 2 of *F. oxysporum* f. sp. *pisi*, new strains or races of *Fusarium* wilt are evolving that cause severe economic losses in race 1 resistant peas. Cultivars are currently available with resistance to races 1, 5 and/or 6, but combined resistance to all races is not currently available in an acceptable commercial cultivar. By determining the predominant race in a given field, the appropriate resistant cultivar can be selected for that field. However, epidemiology and symptoms of the disease caused by races 1, 2, 5 and 6 on susceptible cultivars are indistinguishable Haglund *et al.*, (1970). Consequently, an accurate classification of the predominant race of *Fusarium* wilt in each field is dependent upon a soil-sample bioassay to determine the dominant race of the wilt *Fusarium* sp. in that field and *Aphanomyces euteiches*, 125 mg per liter of Dexon (paradimethylaminobenzenediazo sodium sulfonate) is thoroughly mixed with each soil sample. Differential cultivars of peas Haglund (1974) are grown for 30-40 days at 16-22 C in 10 cm plastic pots of the collected soil and then examined for wilt symptoms.

***Fusarium* wilt of Lentils (*Lens esculenta*)**

On a worldwide basis, the most serious *Fusarium* disease of lentil (*Lens esculenta*) appears to be Fusarium wilt cause by *Fusarium oxysporum* f. sp. *lentis* Claudius *et al.*, (1973), Kovacikova *et al.*, (1974) and this discussion will be limited to this disease. Although the disease has not been important in the United States, Vasudeva and Srinivasan (1952) reported that wilt of lentils is a serious constraint on production in India. Symptoms consist of leaf curling that begins on the lower leaves and extends upward, followed by a general collapse and death of the plant. The disease is reported to be most severe when soil moisture levels approximate 25% with a temperature range of 17-31 C.

Vasudeva and Srinivasan (1952) reported that *F. oxysporum* f. sp. *lentis* is host specific and will not infect several other legumes tested. In culture the fungus is indistinguishable from other formae speciales of *F. oxysporum.* Macro-and microconidia are abundant, as are chlamydospores. Optimum growth in *vitro* is 27-30 C and test isolates of *F. oxysporum* f. sp. *lentis* can be classified into eight different groups as determined by morphological and cultural characteristics Dhingra *et al.*, (1974).

Resistance to *F. oxysporum* f. sp. *lentis* exists in cultivars and breeding lines in India Khare *et al.*, (1970) and Russia Kavacikoua *et al.*, (1974). However, reports on the genetics of resistance or the presence of biotypes or races of *F. oxysporum* f. sp. *lentis* were not found. Kamaiyan and Nene (1975) reported that some cultivars of lentils are more resistant in the seedling stage, while others are more resistant in the mature plant stage.

Mehrotra and Claudius (1973) found that pre-soaking lentil seeds in an 80 ppm concentration of Zn and Mn salts reduced *Fusarium* wilt. Also, late-sown (November-December) cultivars wilted less than earlier-sown cultivars due to soil temperature differences. Claudius and Mehrotra (1973) found that root exudates from 21-day-old lentil plants were inhibitory to spore germination of *F. oxysporum* f. sp. *lentis*. This inhibitory root exudate from 21-day-old plants was thought to partially explain why older plants are more resistant than seedlings when artifically inoculated with the fungus.

A number of Indian worker conducted studies in different part of the country such as : Sadasivan (1961) states : "Despite the many approaches that have been made to the problem of the cause and effect of wilt pathogenesis, we have a long way to go before we understand the many complex situations in the sequence of events that culminate in the syndrome". This holds true even now despite the fact that many reviews have been written on the subject. The literature in this area includes the reviews and articles of Buddenhagen and Kelman (1964) : Biological and physiological aspects of bacterial wilt caused by *Pseudomonas solancearum,* Dimond (1955) : Pathogenesis in the wilt diseases; Dimond (1967) : Physiology of the wilt diseases; Sadasivan (1964) : Physiology of wilt diseases; Subramanian and Saraswathi Devi (1959) : Water is deficient; Wood (1967) : Physiological Plant Pathology; and Dimond (1970) : Biophysics and biochemistry of the vascular wilt syndrome. There are also articles by Ludwig (1960). Beckman (1964), and Gaumann (1957, 1958).

TOXIN PRODUCED BY THE FUSARIA

There is an enormous amount of literature on toxins and their role in plant disease. Some of the important contributions in this area are by Gaumann (1954), Ludwig (1060), Braun and Pringle (1959), Deverall (1964), Wheeler and Luke (1963), Dimond and Waggoner (1953) on vivotoxins; host specific plant toxins have been discussed by Pringle and Scheffer (1964); and toxins and plant diseases have been discussed by Kalyansundram and Charudattan (1966) and Husain and Janardhan (1976). Toxins in plant diseases, their structure and mode of action have been ably discussed by Owens (1969). Wood et al., (1972) have compiled papers on phytotoxins and plant disease. Host specific toxins in relations to pathogenesis and disease resistance have been discussed by Scheffer (1976), and non-specific toxins have been discussed by Rudolph (1976). Phytotoxins produced by plant parasites are reviewed by Strobel (1974). Toxins produced by phytopathogen bacteria have been discussed by Patil (1974).

Although the germ theory of disease was known earlier to the science of plant pathology Prevost, (1807) than to human pathology, yet the role of toxins in infections and in diseases was first established in human medicine. The term toxin in plant pathology has actually been adopted from the conceptual sphere of human medicine. The idea that pathogenesis might be due to the production of poisons by the pathogens is by no means new, but it is only recent years that it has been put on a scientific footing.

De Bary (1886) showed that plant pathogens produce toxins but experimental evidence to support this hypothesis was available only in 1913 (Hutchinson,1913). The credit for the discovery of toxin is generally given to Roux and Yersin (1888) who first visualized the presence of a toxin in the disease caused by *Corynebacterium diptheriae*. In the diseased animals he observed symptoms very far from the seat of infection. He conceived that this may not be directly due to the microbe it self but its metabolic product which gets translocated and reveals symptoms at distant place from the actual site of infection.

Wheeler (1975) visualizes toxins as injurious substances produced by organism. According to him, the term is generally restricted to substances active at physiologically low concentrations. Beyond these basic characteristics, there is little agreement on how toxins, with or without modifying prefixes, should be defined. Some follow the practice of excluding enzymes and growth regulators; others believe that one or both of these should be included in the toxin category. Still others restrict the term toxin to low molecular weight substances or to substances produced by micro-organisms.

Different type of Toxins produced by *Fusarium*

1. Lycomarasmin

Much of what we know about small molecular weight toxins and *Fusarium* diseases has come from the Zurich school founded by Ernst Gaumann. One of the earliest substances to be implicated in wilt diseases was the dipeptide lycomarasmin Gaumann, (1951). Although *F. oxysporum* f. sp. *lycopersici* produces this molecule in culture, substantial yields (300 mg liter^{-1}) are only produced in the staling phase after 40 days. Tomato plants develop symptoms of infection within 7 days, but the concentration of lycomarasmin in culture filtrates at a corresponding time is no higher than 10 mg liter^{-1}. Much controversy has centered around the role of this molecule as an in *vivo* toxin.

Treatment of tomato shoots with relatively high concentrations of the purified lycomarasmin induces a rapid upward rolling of the leaflet followed by interveinal necrosis. Leaf laminae undergo rapid desiccation but do not wilt. An important feature of lycomarasmin is that it forms a water soluble chelate complex with iron that increases 10-fold its toxicity to tomato shoots. This iron chelate also causes increased permeability of epidermal cell protoplasts of *Rhoeo discolor* Gaumann and Bachmann, (1957). The evidence in favor of lycomarasmin as an *in vivo* toxin, however, is weak or nonexistent. Notwithstanding the increased toxicity of the iron chelate, a relatively massive dose of lycomarasmin (150 mg

kg^{-1} fresh weight) is required to induce symptoms, and these do not closely resemble those that develop from natural infection. Moreover, the transpiration pattern of tomato shoots following treatment with 10^{-2} M lycomarasmin (2770 ppm) is quite different from that described for the disease by Scheffer and Walker (1953). A further point against its involvement is that it chelates more readily with copper than iron, but the Cu-lycomarasmin complex is nontoxic. Diamond and Waggoner (1953a) showed that whereas the chelating agent, 8-hydroxyquinoline, stopped the toxicity of lycomarasmin fed to tomato leaves, it had no effect on inoculated plants. They also calculated that the potential *in vivo* production of lycomarasmin is only about one-eighth of that required to produce symptoms. Because lycomarasmin has never been extracted from a diseased host, its involvement in symptom induction is tenuous. It could be argued that *in vivo*, the host substrate might act as an inducer to stimulate the production of biologically effective concentrations that were rapidly metabolized, but the time of appearance of lycomarasmin in culture filtrates mitigates against its involvement, at least in the early phase of pathogenesis, Dimond (1955).

2. Fusaric Acid

Fusaric acid (5-n-butyl picolinic acid) is produced as a cultural metabolite by several formae speciales of *Fusarium oxysporum*, but unlike lycomarasmin, it has also been detected in diseased plants. Fusaric acid was first described as a wilt toxin from *F. oxysporum* f. sp. *lycopersici*, *F. oxysporum* f. sp. *vasinfectum*, and

Gibberella fujikuroi by Gaumann et al. (1952). Dehydrofusaric acid (5-butylene picolinic acid) and other related toxic substances frequently occur with it, Kern (1972). *F. oxysporum* f. sp. *lycopersici* produces substantial quantities of fusaric acid : 200 mg liter^{-1} after 20 days in still cultures or 6 days in shake cultures.

A strong argument in favour of the involvement of fusaric acid (unlike lycomarasmin) in pathogenesis has been its detection in plants inoculated with different *Fusarium* sp. Chandramohan and Mahadevan (1968) detected it in cotton only 12 hr after inoculation with *F. oxysporum* f. sp. *vasinfectum*. Working on the same host pathogen combination. Lakshminarayanan and Subramanian (1955) and Kalyanasundaram and Venkata Ram (1956) detected 17 μg of fusaric acid per gram fresh weight of cotton tissue 2 to 3 weeks after inoculation. Similarly, workers have reported its presence in other *Fusarium* infected hosts, e.g., tomato (*Fusarium oxysporum* f. sp. *lycopersici*) Kern and Kluepfel (1956) and banana (*F. oxysporum* f. sp. *cubense*) Page, (1959). Davis (1969) first found the toxin in flax, tomato, and watermelon 3 or 7 days after inoculation with their particular formae. He further found a positive correlation between virulence of six isolates of *F. oxysporum* f. sp. *niveum* and the quantity of fusaric acid produced in live watermelon seedlings. A similar result was found by Kern (1972) with *F. oxysporum* f. sp. *pisi* in peas. A virulent isolate of race 2 of this pathogen produced 80 mg of fusaric acid per liter in culture fluid and 100 to 150 μg g^{-1} in fresh pea

tissue, respectively, whereas a weakly pathogenic strain produced none in the culture or in the diseased plant.

Some additional support for the importance of fusaric acid has been obtained from its production on sterilized resistant and susceptible tissues. When the resistant tomato cultivar Tuckswood was surface sterilized and inoculated with a virulent strain of *F. oxysporum* f. sp. *lycopersici*, approximately 100 μg of fusaric acid was produced per gram fresh weight, whereas a non-pathogenic strain produced none Kern, (1972). Trione (1960) found that *F. oxysporum* f. sp. *lini* produced fusaric acid on susceptible but not resistant flax stems sterilized with propylene oxide (and therefore killed). The pathogen, however, grew with equal vigor on both cultivars. Davis (1969) found that on autoclaved watermelon seedlings three pathogenic strains of *F. oxysporum* f. sp. *niveum* formed 36 to 56 μg of fusaric acid per seedling, whereas a moderately and two weakly virulent strains produced 5 μg or less. The results of Trione (1960), which have subsequently not been repeated, contradict the generally held view that resistance to wilt fungi is a function of living tissue.

Fusaric acid is a weak monobasic acid with a dissociation constant of 1.8×10^{-5} M. At pH values of 4.3, 6.0, 6.8 and 8.7, the percentage dissociations are 2, 50, 86 and 100, respectively. Gaumann (1957) attempted to explain the concentration and localized effects of fusaric acid in the tomato based on the pH of the cell sap of different tissues. Stem and leaf damage was pronounced at pH 4.3,

but only leaf damage occurred at pH 6.2 Gaumann et al., (1952). On this basis, shoots fed with low concentrations of the toxin would show leaf rather than stem damage. The pH for most tomato xylem bleeding sap, however, is in the region of 6.0 to 6.9.

The pattern of fusaric acid injury to plants is of vital importance in interpreting the role of this substance in pathogenesis. At the cellular level, fusaric acid, dehydrofusaric acid, and their pyridine derivatives affect the water permeability of plant protoplasts. Epidermal cells of *Rhoeo discolor* are affected at 10^{-9} M, *Spirogyra nitida* at 10^{-8} M, and medullary cells of tomato at 10^{-7} to $5x10^{-8}$ M, Bachmann (1956, 1957); Gaumann (1958). Linskens (1955) showed an increase of cations and amino acids on the cuticular surface of fusaric acid treated plants, K, Na and Ca increased 345-, 40-, and 7.6- fold, respectively, over controls, and there was also a three-fold increase in amino acids. This presumably reflected enhanced polar leakage of the tissue. No differences in sugars were found.

3. Other Toxins of *Fusarium oxysporum*

Numerous workers have described culture filtrate "toxins" from *Fusarium* sp. with attributed wilt-inducing properties. In most cases the postulated toxins were not purified or detected in infected tissue. The use of unfractionated culture filtrates in a primary screen for wilt toxins is of doubtful validity. Frequently the

noninoculated medium itself is a potent wilt toxin, and in many studies the possible effects of residual salts and sugars were never eliminated. Similarly, when low molecular weight toxins have been suspected, the crude culture filtrate used has almost certainly included pectolytic enzymes and other proteins and large molecules.

Fusarium oxysporum f. sp. *niveum*, causal organism of watermelon wilt, produces, in addition to fusaric acid, a toxin of molecular weight 426 with the trivial name of phytonivein. It has been detected both in *vitro* and *in vivo*. This toxin is a sterol (empirical formula; C_{29} H_{46} O_2), and was shown by Hiroe and Nishimura (1956) to induce irreversible wilting in watermelon shoots at 10^{-5} M. (4.26 ppm). Phytonivein is thermostable, dialyzable, and nonvolatile, and, like fusaric acid, chelates metallic cations. The relatively low effective wilt-inducing concentration and the good correlation between the pathogenicity of different strains and their toxin producing properties make phytonivein a good candidate for an *in vivo* toxin.

Grafting experiments have been used by at least two workers to demonstrate toxin action. Davis (1954) using intergeneric grafts between resistant scions and susceptible tomato stocks, showed that the stock became invaded with the pathogen while the scion remained resistant to colonization. The resistant scions, however, all showed vascular browning and wilting, indicating action of a toxin. In an extension of this work, Keyworth (1963, 1964) claimed that in

intervarietal grafting with resistant and susceptible plants, the resistant scions responded more (i.e., hypersensitively) than susceptible ones to the toxins produced in the stocks. Keyworth (1964) suggested that this was a possible mechanism for the induction of resistance. The toxin in this work is unknown, but it has a low molecular weight and remains in the purified filtrate after fusaric acid has been removed.

SURVIVAL STUDIES

For many *Fusaria*, survival in soil depends on chlamydospores that have the ability to withstand adverse environmental conditions, Nash et al., (1961). Selective media for isolation of *Fusaria* from soil, Bouhat et al., (1971), Komada, (1975), direct observation methods using soil smears and membrane filters, Nash et al., (1961), Old et al., (1973), electronmicroscopy, Old et al., (1973), Van et al., (1976), and autoradiography, Griffin et al., (1974) all have been used to study chlamydospore formation and survival in soil. Most attention has been paid to chlamydospores of the plant pathogenic species, namely *Fusarium solani, F. oxysporum* and *F. roseum*. Three major phases in the life cycle of chlamydospores can be distinguished; formation, dormancy and germination. This paper discusses the origin of chlamydospores in field soil, the factors that induce chlamydospore formation, the ultrastructure of their formation, their lysis during dormancy, and the germination of chlamydospores as far as it affects their formation and longevity. It also discusses the consequences of cultural practices for their formation and survival. Finally, a definition of the term chlamydospore for Fusaria is proposed.

Chlamydospore Formation in Field Soil

Chlamydospore formation in pathogenic Fusaria commonly takes place in hyphae in the infected and decaying host tissue, Christou et al., (1962). Nash et al.

(1961) observed that the site in the cortex where chlamydospores are formed affects their morphology and physiology; those formed on the surface of stems and roots are mainly large structures rich in oil droplets with thick warty walls, while those formed in the intercellular space of the outer cortex are small with smooth, thinner walls. Chlamydospores may also be formed abundantly from macroconidia that originate from sporodochia on lesions at the soil level, Christou et al., (1962), Nash et al., (1961) ; when washed into soil, the macroconidia convert quickly to chlamydospores that are formed either at germ tubes or by transformation of the macroconidial cells, French et al., (1966), Nash et al., (1965). The importance of asexual sporulation in contributing to survival in soil may depend on the host. In the Pacific Northwest U.S.A., oats infected with *F. roseum* f. sp. *cerealis* 'Culmorum' support greater asexual sporulation and thus contribute to higher propagule densities under field conditions than does infected wheat, Cook, (1968).

The possible role of inorganic salts in stimulating chlamydospore formation in conidia was studied by Hsu and Lockwood (1973). Germlings of macroconidia of *F. solani* f. sp. *phaseoli* on membranes floating on water readily formed chlamydospores, in contrast to non-germinated macroconidia, which, in distilled or phosphate-buffered water, did not produce chlamydospores unless inorganic salts were added, Hsu et al., (1973). Of several weak salt solutions, Na_2SO_4 solution was the most comparable to soil extracts and soil in stimulating numbers of chlamydospores to form. Na_2SO_4 did not increase chlamydospore production from

germlings on membrances on water. The environmental conditions most favourable for chlamydospore formation apparently differ according to whether the macroconidium is germinated or not germinated. The beneficial effect of inorganic salts, e.g., $MgCl_2$, on chlamydospore formation from macroconidia of *F. solani* f. sp. *radicicola* and *F. solani* 'Coeruleum' had also been noticed by Griffin, (1965). The close resemblance of chlamydospore formation in weak salt solutions to that on soil and in soil extracts led Hsu and Lockwood (1973) to conclude that an environment deficient in energy, but with an appropriate weak salt solution, may be all that is required for chlamydospore formation.

In contrast to the effects of carbon sources, the reported effects of nitrogen on chlamydospore formation are contradictory. At low macroconidial densities in salt solutions, inorganic nitrogen did not inhibit chlamydospore formation in *F. solani* f. sp. *radicicola* and *F. solani* f. sp. *phaseoli*, Griffin (1976). Schippers (1972) found NH_4Cl and nitrate, but especially the former, to inhibit chlamydospore formation in *F. solani* f. sp. *cucurbitae* in liquid culture as well as in soil at relatively high conidial densities. Conidial densities appear to be a complicating factor in experimental systems used to study chlamydospore formation. At high conidial densities, macroconidia in *F. sulphureum* did not germinate, but every conidium converted into a chlamydospore. At low conidial densities, the conidia germinated but did not convert into chlamydospores, Schneider et al., (1974). The failure of macroconidia of *F. sulphureum* to

germinate at high densities apparently resulted from the presence of a self-inhibitor. Self-inhibitors also may explain the reduced formation of chlamydospores from macroconidia of *F. solani* f. sp. *cucurbitae* at higher densities in soil, Old et al., (1973). Macroconidia of *F. sulphureum* from older colonies converted more readily into chlamydospores than those harvested from younger ones, Schneider, (1974), suggesting that age of macroconidia is also important.

Survival of Chlamydospores in soil

The significance of chlamydospores for survival of pathogenic *Fusaria* in agricultural soils varies widely among species and formae speciales and apparently depends on soil characteristics and climate factors. Sitton and Cook, (1976) observed that chlamydospores from Graminearum are much more sensitive to rapid drying and high temperatures than those of Culmorum. This may explain why Graminerum is rarely recovered from the dryland areas of the state of Washington (USA). Such a difference in persistence in soil between chlamydospores that are morphologically similar has been documented by Nash and Alexander for *F. solani* f. sp. *cucurbitae* and *F. solani* f. sp. *phaseoli*, Nash et al., (1965). Alexander, (1964) obtained evidence with electron microscopy that chlamydospore walls of *F. solani* f. sp. *cucurbitae* formed in soil extracts were thinner than those of the bean *Fusarium* and therefore might be less suited to survival. Van Eck, (1976) observed no differences in wall thickness between

chlamydospores of these two fungi formed in soil, except for wartiness of the outer layer. The warts, however, are rapidly degraded by soil micro-organisms and do not contribute to persistence of the chlamydospores in soil. Instead, he noticed that chlamydospores of *F. solani* f. sp. *cucurbitae* contained relatively little stored lipids compared with chlamydospores of the f. sp. *phaseoli* and *pisi*. To test the relevance of stored lipids to persistence, macroconidia of *F. solani* f. sp. *pisi* were produced on a basal medium containing 2% and 1/64% sucrose, respectively. Chlamydospores formed in soil from these macroconidia had 50% and 25% of their cell contents occupied by liquid bodies, respectively, yet they did not differ in persistence in soil over the first two months after their formation, Van, (1978).

In order to find out that the soil micro-organisms that degrade cell walls of living chlamydospores play an important role in destruction of chlamydospores, Old and Schippers, (1973) noticed with electron microscopy that sloughing off of outer cell wall layers during chlamydospore formation in soil was closely correlated with the presence of soil micro-organisms. Van Eck and Schippers, (1976), however, observed identical processes during chlamydospore formation of *F. solani* f. sp. *cucurbitae* in sterile cultures. Warts on the chlamydospores in soil were readily invaded by soil micro-organisms; perforations of the walls, on the other hand, were seldom observed, Van, (1976). Although the isolated chlamydospore cell wall, except for the electron-dense layer, is readily solubilized by β (1-3) glucanase, in soils amended with chitin and laminarin [β (1-3) glucan],

no perforations or cell wall degradations were observed. Lysis of chlamydospores in these soils was nevertheless enhanced. It was concluded therefore, that under experimental conditions, lysis of chlamydospores is an autolytic rather than a heterolytic process. Reduction in the population density of *Fusarium* in soil after amendment of the soil with chitin to stimulate a chitinoclastic microflora, Buxton et al., (1965), Khalifa, (1965) cannot be ascribed to heterolytic degradation of the chlamydospore wall. It also cannot be explained by increased competition for nutrients, as increased lysis of chlamydospores did not correlate with increase in total numbers of micro-organisms in soil. It is more likely that the increased lysis of chlamydospores in chitin-amended soils, Khalifa, (1965) is due to the toxic effect of ammonia being released during chitin degradation, Schippers et al., (1973).

The term Chlamydospore in *Fusarium*

Based on an elaborate treatise on the origin, structure and function of chlamydospores in fungi, Griffiths, (1974) suggested a definition of the term *chlamydospore* as follows :

Chlamydospore : (Greek = mantle) -- a viable, asexually produced accessory spore resulting from the structural modification of a vegetative hyphal segment(s) possessing an inner secondary wall, usually impregnated with

hydrophobic material, and whose function is primarily perennation and not dissemination.

In *Fusarium*, however, chlamydospore formation may take place in both vegetative hyphal segments and in conidial cells, Christou et al., (1962). General agreement exists on the accretion a new cell wall material during chlamydospore formation. Whether this results in a differentiate secondary wall formation, Campbell et al., (1974) or not, Schneider et al., (1977) is subject to controversy. A double wall" (primary plus secondary wall) often used for the recognition of chlamydospores is also a questionable criterion because primary wall material may disappear Ackly, Old et al., (1973), Van, (1976). On the basis of present knowledge we suggest defining the term chlamydospore for *Fusaria* as follows :

Chlamydospore : -- a viable, asexually produced accessory spore resulting from the structural modification of a vegetative hyphal segment(s) or conidial cell possessing a thick wall mainly consisting of newly synthesized cell wall material; its function is primarily survival in soil.

According to this definition the "changed macroconidia" in *F. roseum* Graminearum described by Nyvall, (1970) and also the thick-walled hyphae such as described by Nyvall and Kommedahl, (1966) have to be considered as chlamydospores. Any thick-walled cell of a *Fusarium* that has survival value, whether or not double-walled, is a chlamydospore.

The present study deals with some factors affective survival of five species of *Fusarium* viz., *Fusarium solani, F. oxysporum, F. equiseti, F. semitectum* and *F. moniliforme*, isolated from soils of different places. Though the effect of various microbiological factors on different soil fungi including ecology and pathogenicity have been studied by many investigators yet a little attention have been paid towards the above aspect. However, many workers including Garrett (1944, 1956, 1963, 1970), Butler (1953), Stover (1953 a, b), Newcombe (1980), Papavizas and Davey (1961), Sen Gupta and Roy (1971) as well as Dhingra and Sinclair (1976) have extensively reported the saprophytic behaviour of fungi in soil.

In the present study, Allahabad, Azamgarh, Mau, Ballia, Ambedkar Nagar and Ghazipur district was selected for survival studies. The soil was dried, and sieved and was infested with 3% maize meal sand culture. This was prepared in 250 ml conical flasks. Rock flask was first filled up by 150 g of sand and maize meal mixture (150 g of dry clean sand + 4.5 g of maize meal) and 20 ml of distilled water was carefully added (100 g dry sand holds 20 ml water at saturation so 20 ml for 150 g sand maize meal mixture gives about 65% saturation). Such flasks were then autoclaved for 30 min at 20 lbs pressure and were inoculated with agar inoculum discs from a colony margin of a 7-10 days old culture of the fungus on potato-dextrose agar. Flasks were incubated for about 4 weeks at 25°C ± 1°C and were shaken after 2 weeks to distribute the fungus.

For the study of the effect of temperature on the survival of the present five species of *Fusarium*, the soil was infested with respective *Fusarium* species in the glass jars. They were incubated at a temperature ranging from 5° to 45°C. After 7 days of incubation, isolations were made an usual. The results were recorded in the Table 5.

The maximum survival of all the *Fusarium* species was observed between 20°C and 25°C. Their number (colonies) decreased as the temperature was either increased or decreased. Maximum number of colonies of *F. equiseti, F. oxysporum, F. solani* were observed at 25°C while in case of *F. semitectum* and *F. moniliforme* maximum number of colonies retrieved was at 20°C. At 5°C and 40°C the survival of the present fungi was minimum. No colonies, however, could be recovered at 45°C.

In the present investigation the effect of soil moisture on the survival of three species of *Fusarium* was assayed in soil in which the water holding capacity was adjusted at different percentage ranging from 10 to 100 percent. The results are recorded in Table 6.

The survival of the present *Fusarium* species was maximum when soil moisture was maintained at 30% water holding capacity. However, above and below 30% water holding capacity of the soil, the survival of *Fusarium* species declined and at 90 to 100% water holding capacity it was almost eliminated.

Stover (1963 a) reported earlier, the optimum growth and survival of five species and forms of the genus *Fusarium* in soil at 15% saturation.

According to Sen Gupta and Roy (1971), in unsterilized soil, maximum saprophytic activity of *Sclerotium rolfsii* occurred at a wide range of relative low moisture (20-50% moisture holding capacity). Similarly Blair (1943) and Papavizas and Davey (1961) reported the effect of soil moisture on the saprophytic survival of *Rhizoctonia solani*. The reduction in population at high soil moisture was attributed by Blair (1943) to a decline in soil creation with an increase in moisture content.

The present study indicated that the five species of *Fusarium* were strongly aerobic and their population can be reduced by maintaining the soil in a saturated condition. This view is also supported by Stover (1953 a).

Garrett (1938 b) reported a decline in the viability of the mycelium of *Ophiobolous graminis* in soils amended with glucose or with other materials having a little or no nitrogen. According to Wrst and Hildebrand (1941), the incorporation of glucose in soil eliminated the fungi involved in straw-berry root-rot.

Soil samples were amended with glucose, sucrose and starch. The different carbon sources were added at a rate equivalent in carbon to that provided by 1% glucose in air dried soil (w/w). Non-amended soils served as control. All soil

samples were then adjusted to 30% water holding capacity of the soil on an ovendry weight basis. Samples were incubated at 22°C ± 1°C for 4 weeks. The results are recorded in the Table 7.

Results from above table show that in general there was marked decline in population *Fusarium* species in soil amended with glucose, sucrose and starch as observed upto 4 weeks.

In soil samples amended with glucose the population of *F. solani, F. equiseti* and *f. semitectum* increased in first week and then declined by the end of 4th week, as compared to control, while there was a continuous decrease in case of *F. oxysporum* and *F. moniliforme*.

Among soils amended with different sources the maximum reduction was recorded with sucrose. The population of all the five species of *Fusarium* declined continuously upto 4 weeks. Starch amended soils had the similar inhibitory effect on the *Fusarium* species population but in case of *F. oxysporum* and *F. moniliforme* the inhibition was a little less as compared to sucrose.

Table : 5

Effect of temperature on the survival of *Fusarium* species

Temperature (°C)	Number of colonies per 50 mg. of soil				
	F. solani	*F. oxysporum*	*F. equiseti*	*F. semitectum*	*F. moniliforme*
5	05	03	07	06	07
10	35	27	31	28	31
15	46	50	58	48	48
20	63	75	70	72	71
25	53	70	78	76	78
30	48	55	68	64	
35	24	21	36	30	28
40	06	02	08	06	04
45	00	00	00	00	00

Table : 6

Effect of soil temperature on the survival of *Fusarium* species

% of water holding capacity of soil	Number of colonies per 50 mg. of soil				
	F. solani	*F. oxysporum*	*F. equiseti*	*F. semitectum*	*F. moniliforme*
10	38	30	34	36	34
20	53	50	51	53	48
30	75	76	78	75	78
40	46	49	44	42	43
50	30	38	35	34	32
60	22	27	25	26	28
70	18	20	17	26	28
80	12	14	16	14	15
90	09	11	10	08	10
100	08	07	06	05	05

Table : 7

Effect of Carbon Sources on the survival of *Fusarium* species in soil

Fusarium species	Treatments	Number of Fusarium colonies after		
		1st week	2nd week	4th week
F. solani	Glucose	98	40	04
	Sucrose	64	42	18
	Starch	32	21	17
	Control	60	61	65
F. oxysporum	Glucose	28	15	06
	Sucrose	09	04	00
	Starch	30	18	10
	Control	75	77	90
F. equiseti	Glucose	80	48	10
	Sucrose	74	15	08
	Starch	30	15	04
	Control	75	51	75
F. semitectum	Glucose	88	58	12
	Sucrose	76	14	07
	Starch	28	12	04
	Control	65	58	70
F. moniliforme	Glucose	26	15	05
	Sucrose	08	04	00
	Starch	36	16	08
	Control	78	75	85

BIOLOGICAL CONTROL

This term means the control of a disease through some biological agency. Garrett defined biological control as any condition or practice under which or whereby survival or activity of a pathogen is reduced through the agency of an other living organism (excepting man himself), with the result that there is a reduction in the incidence of the disease caused by the pathogen. There is an enormous amount of literature available on biological control of plant pathogen. Biological interference with epidemics has been dealt with by Darpoux (1960). The mechanism of biological control of soil-borne pathogens has been reviewed by Ralph Baker (1968) and there are volumes of international symposia covering this aspect (I, 1963; II. 1968; III, 1973 and IV, 1975). The biological control of plant pathogens has been discussed. However, some examples of successful biological control are given here. The first successful control of a root disease by biological means was by Millard and Taylor (1927) who reported control of scab in potatoes caused by *Streptomyces scabies* by green manuring. A saprophytic species or *Streptomyces,* that is, S. *praecox,* develops rapidly on the dead green manure and inhibits the pathogen.

Bliss (1951) successfully controlled *Armillaria mellea* root rot of *Citrus* by fumigating the soil with carbon disulphide. Fumigation of soils with carbon disulphide helped in boosting the population of an antagonistic fungus *Trichoderma viride.* The fumigant kills directly and the fungus *Trichoderma viride* indirectly affects th pathogen Garrett (1958). A very interesting example of

biological control is provided by the work of Rishbeth in England on the control of *Fomes annosus* root disease of pines by a weakly parasitic basidiomycetous fungus *'Peniophora gigantea.* The usual practice in pine plantations is to fell pine trees and the cut tree stump surfaces are painted with Creosote oil. This brought about only partial control because the stump tissue, with progressive drying, cracked, causing fissures through which fungal spores of Fomes annosus could enter and cause infections. Rishbeth showed that if the paste of oidia of *Peniophora gigantea* is applied on the stump it parasitizes rapidly and kills the tissues, leading the way for the saprophytes to colonize and decompose the stump tissues. These become unfit for any attack by *Fomes annosus* which is a specialized parasite.

Wood and Tveit (1955) obtained good control of *Fusarium nivale* on oats in England with *Chaetomium cochliodes* isolates growing on oat straw and amending the same in the field soil.

Organic amendments have been tried for the control of quite a few soil-borne pathogens. *Phymatotrichum* root rot of cotton is controlled by amending the soil with organic materials which stimulate the germination of sclerotia and thus exhaust the inoculum Mitchell *et al.,* (1941). In the caseof *Gaumannomyces graminis,* it has been suggested that the organic amendments rich in carbon and deficient in nitrogen control the take all of wheat, Garrett (1970). This is explained as follows:

Organic amendments having a high *C/N* ratio result in an increase in the microbial number and activity in the soil. Hence, there is a considerable liberatior

of CO_2 by soil saprophytes which suppresses the pathogenic activity of this fungus. Low nitrogen content in the soil reduces the longevity of *Gaumannomyces graminis* in the soil. There are several other examples where soil amendments have been successfully utilized for the control of root disease pathogens. Snyder *et al.* (1959), showed that the bean root rot was contrclled by barley amendments, that is, the amendments having greater *C/N* ratios controlled bean root rot but if nitrogen was added in the soil, control was nullified. *Fusarium solani* requires nitrogen for germination and penetration and if there is deficiency of nitrogen in the soil, it cannot develop, Cook and Schroth (1965) ; Griffin (1964) ; Toussun *et al.*, (1960). Maurer and Baker (1965) showed that *C/N* ratio greater than 25 significantly suppressed Symptoms of bean root rot.

Rhizoctonia root rot of snapbeans has been controlled by Papavizas and Davey (1960), Papavizas (1963) and Davey and Papavizas (1960, 1963) by amending the soil adjusted to *C/N* ratios with 100-40.

Mitchel and Alexander (1962) reported the control of root rot of bean by manuring the soil naturally infested by *F. solani* f. *phaseoli* with chitin 225 kg/acre. Mitchell and Alexander (1963) showed that only chitinous walled fungi are affected by this treatment.

Some other examples of biological control inc1ude *Phytophthora* root rot of Avocado by amending the soil with alfa-alfa meal of low C/N ratio and root rot of *Piper betel,* Mehrotra and Tiwari (1976) by organic amendments of the soil with corn straw supplemented with nitrogen. Root rot and wilt of *Lens culinaris* by

organic amendments of the soil together with the introduction of antagonists in the soil has been controlled by Mehrotra and Claudius (1972).

Populations of microorganisms show a stable dynamic equilibrium that is remarkably resilient because it is biologically buffered by its own exacting internal competition. Microorganisms compete for nutrients, favorable sites, and oxygen, and they are selected for tolerance of unfavorable conditions of carbon dioxide, pH, water, and other microorganisms. They secrete metabolic materials, some of which (antibiotics) inhibit other microorganisms, others of which stimulate some microorganisms to form essential stages in their life cycles (e.g., Pseudomonas spp. on *Phytophthora cinnamomi*). A microorganism, in its normal life processes, thus affects and is affected by others in its habitat. If it outstrips its nutrient supply, its population is reduced by starvation or antagonists. A fluctuating population density is thus maintained within certain definite limits. This makes for stability, even as a brick is stabilized when it is built into a wall. Biological balance between microorganisms, and between them and their environment, is thus the key to their survival.

An alien microorganism can establish in such a community if it is better adapted to the particular ecological niche than are some of the residents, if it is introduced in such numbers as to temporarily or permanently swamp the residents, if it modifies the environment in some way favorable to itself, or if the natural balance has been so disturbed by man that the environment becomes more favorable to the alien than to the resident.

In undisturbed ecosystems, plant-disease epidemics are unknown or rare, pathogen suppression is usual, and health rather than disease is the norm. Biological control, in this context, is the retention or restoration of such a disease-suppressive biological balance. Usually it is not the original balance, but a new one, stable and compatible with the changed conditions. It is doubtful whether agriculture could have developed if such biocontrol was not common, even under the disturbed conditions of cultivation.

The balance for plant pathogens is achieved through antagonism, "the balance wheel of nature," that operates through competition, parasitism, and antibiosis. Established microbiological associations have developed ecological niches that are interlocking and flexibly buffered. These patterns may, however, be changed by nudging with slight environmental changes, by swamping with large numbers of some microorganisms, or by some "shock" such as application of chemical or heat treatments to soil or addition of organic amendments. Man has emphasized the shock approach, largely by the use of chemicals, and has long exceeded the tolerance limits of biological systems. He is now realizing that subtler nudging will achieve the same results, more slowly but also more permanently.

Plant pathogens are dynamic, adaptable, and display an incredible array of ingenious means of survival. To successfully control pathogens released from this biological balance, man has to be inventive and sagacious. He cannot depend on a single procedure to control a pathogen; rather he must use culture practices, resistance, seed and soil treatments, pathogen-free propagules, chemicals,

sanitation, and purposeful biological control. Biocontrol must not be viewed as a single-shot treatment like a chemical spray, but as part of an integrated control program in which each practice compensates in some way for the deficiencies of the others. Its use is not a justification for abandoning proven practices, but it may provide a useful supplementary means of control.

Biological control must work within the context of biological balance. The soil is more stable than the aerial environment in practically all respects, but it can be slowly altered by many conditions. Because soil microorganisms have become adapted to their relatively stable habitat, slight changes in one or more soil factors may exert a profound effect on them. This is especially true of plant pathogens, which are less tolerant of unfavorable conditions such as heat and antibiotics, and are less adaptable nutritionally than are saprophytes. This principle is applied in pasteurization of milk and in treatment of soil at 60°C for 30 min for nursery use Baker (1970, 1971).

Soils that are biologically "live" (i.e., have a heterogeneous, abundant, and active micro biota) are more likely to exert biological control of an introduced plant pathogen than are impoverished biologically "dead" soils. A live soil has a fairly high content of organic matter and mineral nutrients and is well aerated and moist. It often exerts such an antagonistic effect on pathogens that erroneous deductions may be made about host resistance. For example. on the basis of field observations. it was thought that *Phymatotrichum omnivorum* (cause of cotton root rot) did not attack corn. However. when corn was grown in sterile soil. it was attacked and killed. Similarly. *Gaeumannomyces graminis* var. tritici (cause of

take-all disease of wheat) attacks *Gramineae* but not dicotyledonous plants in the field; in sterile soil it is able to severely attack peas and other plants, Baker and Cook (1974).

Suppressive soils represent a balanced state of the microbiota. stabilized by the interactions of heterogeneous microorganisms. In the two examples intensively investigated thus far (*G. graminis* var. tritici and *Phytophthora cinnamomi*), no single organism of the many extracted from suppressive soils has been found to be the active suppressor. A biologically suppressive soil probably cannot be explained in terms of a single antagonist. Populations antagonistic to a given pathogen occur, only in soils biologically suppressive to it. but in- dividual antagonists occur in many soils, Baker and Cook (1974). These individual antagonists may be quite effective in a one-to-one situation (a single antagonist to a single pathogen in a medium free of other microorganisms). Thus, one should attempt mass transfer of a total antagonist population when nontreated soil is involved, but one may use a single antagonist when introduced into soil or onto a plant surface of near-sterility.

Antagonists are usually nonmobile and generally make accidental contact with the pathogen. Many are specialized inhabitants of the rhizosphere; few are generalized inhabitants of the soil mass.

Elimination of the pathogen from a site is rarely achieved by any method and is not essential for biocontrol. Inhibition of the pathogen or suppression of its pathogenic activities, without overkill of other microorganisms, is the objective. A biological vacuum should be avoided, for the first microorganism to return will

luxuriate; if this is a pathogen. severe disease will result. Nursery and glasshouse soils are steamed at 60°C, rather than 100°C, for 30 min so that many saprophytes survive and buffer any pathogen that may be accidentially introduced, Baker (1970, 1971).

Chemicals also may be used selectively to favor antagonists and/or inhibit or destroy the pathogen. Soil treatment with carbon disulfide has long provided effective control of *Armillaria mellea* in California orchards. The chemical at low to medium dosages so weakens the pathogen that it is then destroyed by *Trichoderma viride.* but at high dosages it kills the pathogen. Methyl bromide inhibits production by *Armillaria* of the toxin that protects it from *T. viride.* Aerated steam treatment also increases the vulnerability of *Armillaria* to *Trichoderma,* Ohr and Munnecke (1974) ; Munnecke et al,. (1976), Tronsmo and Deninis (1978) found that *T. viride* isolates inhibited other fungi by volatile materials at high temperatures, but that temperature had little effect on the production of nonvolatile inhibitors or on hyperparasitism.

The Del Monte Packing Corporation found that soil treatment with carbon disulfide at 3700 kg/ha prior to planting asparagus in California gave poorer immediate control of fusarium wilt (caused by *F. oxysporum* f, sp. *asparagi*) than did chloropicrin (337 kg/ha) but that after several years the total control was better for carbon disulfide.

Soil fumigation with Telone (1, 3-dichloropropene) reduced *Verticillium dahliae* injury to potatoes without reducing the population of the pathogen in the

soil; the mechanism of this effect is not known, Easton et al.. (1975). Likewise. fusarium blight (caused by *F. roseum*) of bluegrass was controlled in New York by application of hydantoin fungicide [1-isopropylcarbamoyl-3 (-3, 5-dichlorophenyl) hydantoin] even though the propagules of the pathogen increased fourfold. Numbers of *Streptomyces* sp.. Bacillus sp. and total bacteria also increased. The control was thought to indirectly affect host metabolism or soil microbial balance rather than to act directly on the pathogen, Smiley and Craven (1977).

The studies discussed in this section suggest that chemicals may achieve disease control by nudging the microbiological balance rather than directly killing the pathogen. This could open a new era of plant disease control, even as sulfanilamide and penicillin changed medical practice from killing human pathogens with poisons to inhibiting them with antibiotics, Hare (1970).

Environmental factors often selectively affect soil microorganisms, and they may be used to direct the interaction between pathogen and antagonists to produce biocontrol. Bacteria grow and develop in moist soil but become dormant or die when the soil becomes moderately dry (-10 to -15 bars water potential). *Fusarium roseum* 'Culmorum' is in- hibited by bacteria in moist soil but causes a severe foot rot of wheat when the soil dries to -75 to -85 bars. Prolonging the moist period of the soil by creating a dust mulch immediately after rain during the summer fallow, by chisel plowing in the fall to increase water infiltration. by production of smaller plants through late seeding and using minimal fertility for a satisfactory yield. and by growing varieties of high water efficiency decrease losses from this disease in Washington, Cook and Papendick (1970). Conversely, *Cephalosporium*

gramineum. cause of a stripe disease of winter wheat, resists bacteria and fungi in moist soil. When the soil becomes very dry (-150 to -210 bars), the pathogen is unable to produce its protective antibiotic and is then susceptible to *Penicillium* spp., which grow at these water potentials, Bruehl and Lai (1968).

Natural hyperparasitism of soil fungi by amoebae has been studied in detail by K. M. Old and associates since 1967. Cell walls or hyaline or pigmented conidia, chlamydospores, and sporangiospores, are of 15 species of fungi, as well as nematodes and their eggs, are perforated and lysed by a resident giant vampyrellid amoeba resembling Leptomyxa *reticulata,* Old and Darbyshire (1978, 1980). The holes are 0.2-0.5 or 0.5-6.0 μm in diameter. Melanin in the spore walls does not protect against the amoeba as it does against other microorganisms, Bull (1970). Dead spores apparently are not attacked. The amoebae engulf, but do not perforate or lyse *Fusarium* macroconidia or the sclerotia of *Botrytis cinerea* and fail to perforate spores of seven other fungi. Oospores of *Phytophthora fragariae* are engulfed and lysed. The amoebae also feed on bacteria. flagellates, blue green algae and diatoms. They are known to occur in England, Canada and Holland. Anderson and Patrick (1977, 1978) found that one amoeba (Vampy-*Tello* sp.) caused perforations 2-5 μm in diameter and that another (*Arachnula* sp.) caused perforations of 1 *p.m* or less in spore walls of *Thielaviopsis* basi *cola* and *Helminthosporium sativum.* Small holes (0.2-0.5 *p.m* in diameter) in fungus spores in soil were thought also to be produced by bacteria aligned end-on to the surface, Old and Wong (1976). It was pointed out by Barron (1978) that Drechsler (1936. 1937) had described perforations of fungus spores by the amoebae *Geococcus*

vulgaris and *Euglypha denticulata.* It thus appears that amoebae of five different genera are able to perforate fungus spores in soil and that the phenomenon must be fairly common in nature.

Because these amoebae are widely distributed and attack many microorganisms, particularly spores resistant to other microorganisms because of thick and/or melanized walls, they are of great interest and potential value in biocontrol. It should be remembered, however, that *Bdellovibrio bacteriovorus.* nematode-trapping fungi. and bacteriophages, Baker and Snyder (1965), also common in soil and undoubtedly important in natural biocontrol, have so far not proved useful in commerical biocontrol of plant pathogens.

Observations, Stirling and Mankau (1978) in a peach orchard in the San Joaquin Valley, California. showed that old trees on Lovell rootstocks, which are highly susceptible to root-knot nematode, were growing well despite the presence of *Meloidogyne* sp. Because of this unusual situation the orchard was studied. The fungus *Dactylella oviparasitica* was destroying nematode egg masses so completely as to provide excellent field biological control. Eggs at all stages of embryological development. including second-stage larvae, were attacked. This is an example of the benefit of finding and studying sites where the pathogen occurs without producing disease (see Section IV,B).

Biological control by a single introduced microorganism may be successfully used when the soil or plant surface is virtually free of other

microorganisms. Some success also has been attained by inoculating nonsterile plant propagules with antagonists.

Soil treated by steam at 100°C/3D min may be inoculated with an antagonist and provide effective biocontrol of *Rhizoctonia solani* and *Pythium ultimum,* Ferguson (1958); Olsen and Baker (1968); Broadbent et *al.,* (1971). Inoculation of the nearly sterile casing soil of commercial mushroom beds with *Pseudomonas multivorans* or *P. fluorescens* has controlled brown blotch of the caps caused by *P. tolaasii,* apparently through competition, Nair and Fahy (1976). The inoculation of Fusarium-suppressive soil into treated soil is under investigation in Colorado and appears promising.

Commercial control of *Heterobasidion annosum* (*Fomes annosum*) of pine on 62,000 ha of forest in England is achieved by inoculating freshly cut stumps with oidia of the low-grade pioneer pathogen, *Peniophora gigantea.* Infection and spread of. *Heterobasidion* to the roots of healthy adjacent trees is thus prevented, Rishbeth (1975); Gibbs and Smith, (1978). This apparently is another example of the possession principle, operating through a short-range antibiotic effect by a primary antagonist. Gibbs and Smith (1978) suggested that strains of *Armillaria mellea* of low virulence, Redfern (1975) might also act as primary antagonists of *H. annosum.*

The primary antagonist, *Trichoderma viride.* inoculated on branch stubs with the pruning shears, has been used for control of the silver leaf disease (caused by Stereum purpureum) on plum trees in France, Grosclaude et *al.,* (1973).

A chemical may be combined with a nonsensitive antagonist to supplement the biological control. *Fusarium lateritium*, insensitive to benomyl, is applied with that fungicide to fresh pruning wounds on apricot trees to protect against *Eutypa armeniacae*, cause of a canker disease in South Australia. The fungicide protects against Eutypa until the antagonist has established in the wound, Carter and Price (1974).

There is substantial evidence that an antagonist inoculated on a plant propagule may prevent infection by plant pathogens, including wilt fungi. Woltz et *al.* (1978) and Magie (1978) inoculated corms of three gladiolus varieties in Florida with *Fusarium oxysporum* f. sp. *gladioli* (cause of yellows) and dipped them in either a spore suspension of F. *moniliforme* 'Subglutinans' M-685 or in a fungicide mixture prior to storage. The average percentages of healthy corms in three experiments when dug were: check 24.3, M-685 52.8, and benomyl- captan 43.5. Treating gladiolus corms with a fungicide was found to be more injurious to resident antagonists than it was to *F. oxysporum* f. sp. *gladioli* and four other fusaria that caused corm rot. Fusarium-infected corms of two varieties planted for 2 years in a suppressive Florida red lateritic clay that had been planted to sugarcane for 150 years and contained many *Streptomyces* sp., produced apparently healthy daughter corms. However, the corms harbored latent *F. oxysporum* f. sp. *gladioli* and rotted the first year when planted in conducive sandy soil. The suppressive clay soil, as expected, had not eradicated the fungus in the corm tissue.

Inoculation of seeds with antagonists may also be an effective means of achieving biological control. Corn seed inoculated with *Bacillus sub-tilis* or *Chaetomium globosum* and planted in Minnesota fields that had a moderate inoculum density of *Fusarium roseum* 'Graminearum', gave about as good control of seedling blight as captan or thiram seed treatment, Chang and Kommedahl (1968); Kommendahl and Mew (1975). The effect was thought to result from antibiotic production by the antagonist that persisted in the rhizosphere, but the antagonist may also have swamped the pathogen inoculum. In similar experiments, Mitchell and Hurwitz (1965) inoculated "sterilized" tomato seeds with a rhizosphere *Arthrobacter* sp. and planted them in treated or nontreated , soil inoculated with *F. oxyspotum* f. sp. *lycopersici.* Wilt was greatly decreased.

Inoculation of the roots of dug seedlings of stone fruits with the avirulent Agrobacterium radiobacter mutant 84 has provided excellent control of crown gall caused by virulent A. *tumefaciens* in widespread tests over the world, Kerr (1974); Moore and Warren (1979).

Inoculation of seeds with specific bacteria has increased subsequent plant growth in numerous tests, even when pathogens were not known to be involved, Broadbent et al., (1977); Burr et al., (1978); Kloepper and Schroth (1978). The causers of such growth increase is still uncertain, but the most effective isolates produce broad-spectrum antibiotics and may be inhibiting nonparasitic microorganisms that decrease plant growth. Work in this important field is expanding the scope of plant pathologists beyond their traditional concern with parasites that penetrate the host and produce disease. Their studies must now also

in- clude microorganisms (nonparasitic pathogens or exopathogens) in the rhizospores that decrease plant growth but rarely or never penetrate the root, Woltz (1978).

Since there is a positive relationship between effectiveness and inoculum density of the antagonist introduced on seeds, seedlings, and plant propagules, pelleting to increase the biotic load has been used, Merriman et al., (1975).

Antagonists must affect either the pathogen or the host if biological control is to be effective. Once wilt pathogens are within the host, they largely escape the effects of antagonists, and biocontrol (with the exception of host resistance) therefore generally has been targeted on the preinfection phase of the pathogen. However, recent studies with avirulent isolates of the pathogen, and studies on the nature of resistance have enlarged this viewpoint.

Although some may question whether host resistance is a form of biological control, the two fields often are found on close study to be so inextricably interwoven that there is no benefit from trying to separate them (see Section II). Disease interactions always involve at least two organisms (the host and the parasite), and frequently also an avirulent strain or a saprophytic microorganism. Resistant varieties may starve a pathogen by the absence of a favorable nutrient base quite as much as does an antagonist or crop rotation.

It is to be expected that plant exudates known to be selectively favorable to soil microorganisms sometimes are a basis of host resistance. However, nonparasitic microorganisms have only recently been shown to be governed by

host genes and, therefore, to be controllable by plant breeding. Atkinson and associates showed, in a series of papers, that "the genotype of the host governs the magnitude and composition of bacterial populations in the rhizosphere with surprising specificity...presumably through its control of the quantity or quality of root exudates, or both", Atkinson et al., (1975). They used wheat varieties resistant ('Apex') and susceptible (S-615) to root rot caused by *Cochliobolus s(Jtivus (Helminthosporium s(Jtivum)* and a resistant hybrid (S-A5B) identical to S-615 except for the substitution of the chromosome pair 5B from' Apex' for its homologue. The total bacterial count of the rhizosphere of S-615 was double that of resistant' Apex' or S-A5B. However, 20%0 of 'Apex' or S-A5B rhizosphere bacteria were antibiotic to *C. sativus*, whereas none from S-615 were. Similar differences were observed with resistant cultivar 'Cadet', susceptible 'Rescue', and susceptible C-R5B. This opens a new aspect of plant breeding for resistance perhaps applicable to wilt diseases, as well as emphasizing its interrelationship with biocontrol.

Conversely, resistant varieties may be rendered susceptible by conditions external to the root. Cotton varieties resistant to *Thielaviopsis basicola* may become susceptible in the presence of hydrocinnamic acid, which may arise from decomposing organic matter in soil (Linderman and Toussoun, 1 968). It was thought that the compound prevented formation of or removed inhibitory materials, increased exudation of stimulatory materials, or both.

However in the present study a number of different type of antagonist of Fusarium infestation has been observed in the lab condition. Attempts has been

done to change the environmental condition so that the agro-climatic condition of Allahabad and adjoining areas could be properly simulated. In our observation it has been remarkably found that biological factor plays a significant role in the cause effect parameter of Fusarial disease in different types of crops. The findings of the present studies have been listed in the table.

Some microorganisms function as antagonists by stimulating host resistance to a pathogen. The mechanism by which resistance is stimulated usually is unknown or not investigated.

Inoculation of flax with an avirulent race of *Melampsora lini* made the leaves resistant to a virulent race subsequently applied, Littlefield (1969). Resistance to *Colletotrichum lindemuthianum* has been induced in bean hypocotyls by prior inoculation with a race avirulent to the cultivar, Rahe et *al.*, (1969). 'Jersey Orange' sweet potato (susceptible to *Ceratocystis fimbriata)* inoculated with any of eight isolates non- pathogenic to sweet potato was resistant when inoculated 2 days later with a pathogenic isolate. Other *Ceratocystis* spp., *Verticillium albo*utrum, or Phytophthora *infestans* induced no resistance, Weber and Stahmann (1969).

When cuttings of susceptible tomatoes were placed in suspensions of different proportions of microconidia of *Fusarium oxysporum* f. sp. *lycopersici* and f. sp. *pisi* before planting, wilt became less severe as the ratio of *pisi* was increased. Heat-killed spores of *pisi* did not produce the effect, Langton (1969). Similar results were obtained with *F. oxysporum* f. sp. *melonis* when muskmelon

was inoculated simultaneously with virulent and nonvirulent isolates. Protection was explained as resulting from competition for nutrients and space. When a *Cephalosporium* sp. from roots of healthy tomato plants was inoculated into tomato seedlings prior to inoculation with *F. oxysporum* f. sp. *lycopersici,* the longer the period between the two inoculations the greater was the protection. Repeated isolation of inhibitory strains from healthy survivors apparently intensified the protective effect.

Inoculation of the cut surfaces of sweet potato cuttings with isolates of *F. solani,* a low-grade pathogen that occupied the wounded surfaces, prevented infection by *F. oxysporum* f. sp. halalas and controlled fusarium wilt, McClure (1951). Inoculation of the freshly cut surface of carnation cuttings with *Bacillus subtilis* or Pseudomonas sp. protected them from fusarium stem rot (caused by *F. roseum* 'Culmorum' when rooted and planted in infested soil. An *Arthrobacter* sp. was used in a slurry of milled lobster shell to inoculate roots of carnation cuttings before planting in soil inoculated with *F. roseum* 'Culmorum'. The bacteria lysed the mycelium only when other carbon sources were not available; they attached to the *Fusarium* mycelium, perhaps because of the chitin in the fungus walls.

Dick (1974) found that a tomato cultivar resistant to *Fusarium oxysporum* f. sp. *lycopersici* inoculated with that pathogen became resistant to *Verticillium dahliae.* Similarly, mint inoculated with the weakly pathogenic *V. nigrescens* 2-9 days before inoculation with the pathogen *V. dahliae* became progressively more resistant to it (Melouk and Horner, 1975). Roots of tomato seedlings were dipped in spore suspensions of avirulent *V. alboatrum, V. tricorpus, Fusarium oxysporum*

f. sp. lycopersici, or *F. oxysporum* f. sp. dianthi and planted in glasshouse benches of soil infested with virulent *V. dahliae*. However, only a fair degree of control of veriticillium wilt was obtained under commercial conditions.

The vesicular-arbuscular mycorrhizal fungus, *Glomus fasciculatus*, increased the severity of verticillium wilt of cotton (caused by *V. dahliae*) in California over noninoculated plants. It was concluded by Schenck and Kellam (1978), in a literature review of the influence of mycorrhizae on fungus, bacteria, nematode, and virus plant pathogens, that vesicular-arbuscular mycorrhizae increased disease severity in seven instances, decreased it in 20, and had no effect in nine. They cautioned against commercial use of these mycorrhizae as "biological fertilizers" in soils containing pathogens until this phenomenon is better understood.

Although several of these studies appear to have commercial promise for biocontrol of important pathogens, none is known to have been commercially used. This may be because of the risk that an avirulent microorganism may mutate to become virulent to the same or another host or may act synergistically with other pathogens. However, few studies have been carried for enough either to confront these risks or to be tested in commercial fields. More likely, such studies have reflected the tendency, Baker and Cook (1974) to regard biocontrol research as academic laboratory and glasshouse exercises rather than as purposeful efforts to control diseases in field situations. The emphasis seems to have been on publishable papers rather than on aiding agriculture.

Resistance appears to result in some cases from the natural occurrence of antagonists rather than from plant genetical factors. Oat varieties from Brazil were resistant in Minnesota to *Helminthosporium victoriae* in the field, Tveit and Moore (1954). This resistance was due to the antagonists *Chaetomium globosum* and *C. cochlioides*, which occurred naturally on oat seeds from Brazil, where *H. victoriae* is unimportant. Hot-water treatment of the seed killed the antagonists and made these Brazilian varieties fully susceptible, Wood and Tveit (1955). Resistance to wilt fungi might also be caused by seedborne antagonists.

The stimulation by microorganisms of resistance to wilt pathogens through vascular occlusions and gel formation, is another way in which pathogens or nonpathogens may act in host resistance. Indeed, for many diseases there is little benefit from trying to separate host resistance and biological control.

If vascular parasites were commonly transmitted internally through seed, the life cycle would be essentially a closed circuit. The pathogen would not be exposed to the hazards of finding and infecting a host while exposed to the *vicissitudes* of the physical environment and antagonists. Such transmission may be rare because, in an evolutionary sense, it was too successful, too many hosts were killed, and such pathogens perhaps became extinct. In any case, *Verticillium alboatrum* and *V. dahliae* are internally seed transmitted only in achenes of sunflower, *Senecio*, spinach, and safflower, and in burs of *Xanthium*, Baker (1972). The fruit coats of achenes and burs have more extensive vascularization than do seed coats, and vascular parasites thus have a better chance of transmission. Furthermore, successful seed transmission of *Verticillium*, as well as

other pathogens, is strongly reduced when seed is planted in nonsterile soil, due to the antagonistic effect of microorganisms, Baker (1972).

Fusarium oxysporum f. sp. *mathiolae* spreads into seed through the vascular elements and is internally transmitted, Baker (1948), a situation apparently rare in vascular fusaria. This rarity is probably part of the reason why suppressive soils are so effective against the vascular- wilt fusaria. It has also been suggested, Watson (1970) that competition of these pathogens with soil saprophytes retards or prevents population shifts and thus stabilizes effective host resistance.

Certain soils are so inhospitable to some plant pathogens that they cannot establish in the soil, they may establish but produce no disease or they may establish but the disease diminishes with continuous culture of the crop. These categories are man-made. and intergrades probably exist in nature. As J. E. Van der Plank noted, "Nature seldom draws lines without smudging them:" These categories are, however, useful indicators of effective biological control in the field-instances where the pathogen is or has been, but the disease does not occur. When biological control is found to be naturally working, it should be thoroughly investigated with the objective of extending its use to other situations.

Suppressive soils are quite common. but usually not recognized as such. One can be recognized only when a suceptible crop and a pathogen are brought together in it. When the host environment-pathogen-antagonist complex favors the occurence of disease, it is recognized by the symptoms produced-a positive observation. If antagonists suppress the activity of the pathogen. there are no

symptoms of disease-a negative observation that easily is dismissed as due to the absence of the pathogen or to a physical environment unfavorable for disease. However, man has unconsciously made extensive agricultural use of suppressive soils.

Fusarium oxysporum f. sp. *pisi* (cause of *fusarium* wilt of peas) has long been introduced into California on the seed of susceptible pea varieties extensively planted for canning, freezing, seed production, and the fresh market. In only two small coastal areas has the wilt disease ever developed, even though susceptible pea varieties have been widely grown; these areas are now subdivided for homes.

Suppressive (resistant, long-life) soils that required 20 years of banana cultivation for Panama disease (caused by *F. oxysporum* f. sp. *cubense*) to become severe were found by Reinking and Manns (1933) in Guatemala, Honduras, Costa Rica, and Panama. Bananas planted in conducive (nonresistant, short-life) soils would succumb in 3-4 years. The long-life soils developed only low populations of the pathogen, and high disease incidence was associated with high populations of the pathogen. Use of this classification of Central American banana soils was an important factor in controlling Panama disease there until a shift was made from the susceptible 'Gros Michel' to resistant cultivars summarized the-work on Panama disease of banana, much of it not previously published. In general, long-life soils were clay, and short-life soils were sands. In a series of papers from 1961 to 1967, Stotzky and associates showed that montmorillonite clays were present in long-life banana soils and lacking in short-life soils. The proportion of montmorillonite present generally was directly related to the degree of

suppression. These clays were found to stimulate bacterial activity, perhaps checking growth of the *Fusarium*.

Nelson (1950) briefly reported for *Verticillium albo-atrum* var. mentho on peppermint in Michigan that "some soils resist invasion by the wilt fungus while others apparently do not This resistance was destroyed by steaming." When mint rhizomes from fields with severe wilt were planted in these suppressive soils. it was several years before the disease appeared. In conducive soils the disease appeared promptly and severely. Apparently this is the only report of *Verticillium*-suppressive soil. Since this pathogen is so sensitive to the presence of pected that soils suppressive to it would be fairly common.

Although the Salinas Valley, California, has extensively grown a wide variety of crops for many years, fusarium wilt diseases do not occur there. However, they do occur in the adjacent Castroville area. Smith and Snyder (1971) found in glasshouse tests that *F. oxysporum* f. sp. *batatas* inoculated into these soils produced less severe wilt in the suppressive Salinas than in the conducive Castroville soil at 50, 500, or 5000 chlamydospores soil. In the series with 500 chlamydospores/g, wilt was more severe 8 weeks later in conducive than in suppressive soil. The number of propagules remained the same in suppressive soil, but doubled in the conducive soil, compared the behavior of *F. oxysporum* f. spp. *batatas cubense*, and *lycopersici* with a closely similar saprophytic *F. oxysporum* from the suppressive soil in each case. When they were inoculated in suppressive and conducive soils and nutrients were added, germination of chlamydospores was lower and the germ tubes shorter in the suppressive than in the conducive soil.

Saprophytes had higher percentages of germination and longer germ tubes than the pathogens in both soil types. An *Arthrobacter* sp. increased more rapidly in the suppressive than in the conducive soil when nutrients were added. Suppression clearly was specific in that it acted against the pathogens but not the saprophytes native to that soil: Smith (1977) found that germination of chlamydospores, and hypha I growth of *F. oxysporum* f. spp. vasinfectum and *tracheiphilum* were less in suppressive than in conducive soils. Hyphal growth ceased after 24 hr in suppressive soil, but continued for 60 hr or more in conducive soil, a time sufficient for infection of the host. An *Arthrobacter* sp. was associated with the germlings in suppressive, but not in conducive soils, and increased in numbers as the germ tubes grew. The numbers of *Arthrobacter* increased in suppressive soils to which *Tracheiphilum* chlamydospores were added. Inhibition or lysis of *Fusarium* mycelium by *Arthrobacter* sp. was slight on agar plates; *Bacillum* sp. and *Actinomycetes*, however, caused extensive lysis. This is in contrast to the extensive lysis of f. sp. *lycopersici* by the *Arthrobacter* observed by Mitchell and Hurwitz (1965) in culture. Although these bacteria would be effective only in environments in which they could proliferate, in the suppressive soils studied they even protected plants growing under stressed conditions. The bacteria may not be able to grow if introduced into conducive soils unless means are found to make the medium favorable to their growth.

The evidence strongly suggests that a soil suppressive to one forma specialis of *Fusarium* oxysporum will be suppressive to its other formae speciales as well. This indicates that these formae speciales are closely similar in

characteristics that affect their interaction with the suppressive microbiota. However, this specific suppression does not extend to *F. roseum, F. sol ani* or to saprophytic *F. oxysporum,* although other soil micro biota may be suppressive to them. On the other hand, the host strains of *Rhizoctonia solani* appear to be sufficiently distinct that microbiota in soil suppressive to the wheat strains are not suppressive to the crucifer strain.

Phytophthora cinnamomi has been present for nearly 40 years in an avocado grove on Tamborine Mountain in Queensland. Australia, under 250 cm or more of rain per year without causing root rot. Steam- ing the soil at *100°C/30* min makes it fully conducive when inoculated with the pathogen. indicating that the suppressiveness is biological in origin. However. the soil remains suppressive after treatment with aerated steam (Baker. 1970, 1971) at *60°C/30* min. showing that the antagonists are thermal-tolerant spore-forming bacteria or actinomycetes. Multiple antagonists are involved. Suppression of the soil can be temporarily lost following waterlogging or flooding. probably because of altered microbiological balance, Broadbent et *al.,* (1971). The Phytophthora-suppressive soils are characteristically high in organic matter and calcium, with the calcium and ammonium nitrogen tied up in the organic cycle. The pH is near neutrality and the microbiota is both abundant and varied, Pegg (1977). Intensive cover-cropping, and applications of lime, poultry manure, and superphosphate, materials now extensively used by Australian avocado growers, have provided satisfactory commercial control in avocado groves.

In another avocado grove badly injured from root rot, replanted, and given the above culture, the pathogen has declined below detectable levels, Pegg (1977). This situation is quite different from the one above, resembling Section IV, C and demonstrating the smudged boundary between Sections IV, B and IV, C.

Pegg (1977) showed that biocontrol of the pathogen on pineapple is possible by a different technique. Since pineapple suffers from iron chlorosis if the soil is kept near neutrality, lime cannot be used for disease control as it is on avocado. Instead, sulfur is added to bring the pH below 5.4. Antagonistic bacteria and nitrifying bacteria are in- hibited under these conditions, and nitrogen remains in the ammonium form, which is somewhat inhibitory to Phytophthora. The pathogen in this crop apparently is controlled under these conditions by *Trichoderma viride*, which is favored by acid soil; *T. viride's* an- tibiotics, gliotoxin and viridin, are stable only at a low pH.

The examples described in this section show that it is not necessary to eliminate the pathogen in biocontrol and that slight edaphic changes may activate resident antagonists to effect disease control. It was reported in Section II. A that *Verticillium* and *Fusarium* may be controlled by chemical treatments that do not reduce the inoculum density. The possibility of using soil amendments and associated crops for a similar effect on vascular wilt pathogens should be investigated.

Vascular pathogens that have breached the host defenses and reached the vascular elements are well-protected from antagonists.

Pseudomonas syringae produces toxins *in-vitro* that are inhibitory to *Ceratocystis ulmi*, the cause of Dutch elm disease, Myers et al., (1978).

It is well known, Kreutzer and Baker, (1975) that some bacteria that are saprophytes or pathogens of low virulence occur quite commonly in the vascular elements of plants, causing minimal injury, particularly to trees. These microorganisms seem to provide no useful defense against vascular pathogens. Is it possible, however, that only pathogens insensitive to these fellow vascular inhabitants are able to produce symptoms and that, were these bacteria not present in the vascular elements, other diseases, presently trivial or not seen at all, might become important ?

These prevalent microorganisms clearly are well-adapted to the vascular habitat, and only certain ones are able to survive there. It is unlikely that random microorganisms isolated from soil and found to be antibiotic to a pathogen in culture, would be able to flourish in the vascular system or to inhibit the pathogen there. Could a bacterium highly antibiotic to the pathogen be made to achieve this desideratum by genetic recombination with one of the resident vascular bacteria ? This might be a better possibility for the control of the Dutch elm disease, for example, than the use of the secondary antagonists suggested in the following paragraph.

The interesting recent discovery of hypovirulent strains of certain pathogens presents a potential for biocontrol that might be applicable to wilt diseases. There have been many reports of degenerative diseases of fungi, seven of them in plant

pathogens [*Helminthosporium victoriae*, *H. maydis*, *H. oryzae*, *Endothia parasitica*, *Gaeumannomyces graminis* var. *tritici*, *Rhizoctonia* solani, and *Colletotrichum lindemuthianum]*. Mycoviruses or virus-like particles have been reported in over 30 species of plant-pathogenic fungi, but altered fungus phenotypes are known to have resulted only in *Saccharomyces cerevisiae*, *Ustilago maydis*, and *Agaricus bisporus* Castanho et *al.*, (1978).

Castanho and Butler (1978a,b) and Castanho et *al.* (1978) found that hypovirulence in *Rhizoctonia solani* was associated with three segments of double-stranded RNA. The "diseased" culture arose from a healthy isolate. In glasshouse tests in treated soil, uninfested or infested with diseased. healthy. or a mixed culture of both fungus types, sugar beet seedlings had 0.05, 0.11, 79.0 and 2.8% damping-off, respectively. In another glasshouse test in which the inoculum was placed in the seed row. damping-off of sugar beet seedlings was 0.27, 76.0, and 15.1 % for uninfested, healthy, and a mixture of diseased and healthy isolates, respectively. Because the diseased fungus did not survive 1 month in sandy loam (as opposed to 2 years for the healthy isolate), it was considered unlikely that it would establish in field soil for biocontrol. To be effective, it was necessary for both the healthy and diseased cultures to be actively growing so that anastomosis and transmission of the factor would occur. Single basidiospores of the healthy isolate may give rise to diseased cultures, showing that the healthy isolate contained traces of the double-stranded RNA. Occasional hyphal-tip isolates from diseased cultures were healthy, but treatment with heat, antibiotics, or acridine dyes did not cure diseased cultures.

The following cultures of antagonists were used for *in vitro* test against pathogenic Fusaria causing wilt, root-rot and seed-rot diseases of crops and plants.

(1) *Aspergillus flavus* Link

(2) *Alternaria alternata* (Fr.) Keissler

(3) *Chaetomium globosum* Kunze ex Fr.

(4) *Chaetomium arcuatum* Rai & Tiwari

(5) *Cladosporium oxysporum* Berke & Curt

(6) *Colletotrichum* state of *Glomerella cingulata* (Stonem) Spauld & Schrenk

(7) *Curvularia lunata* (Wakker) Boedijn

(8) *Fusarium arthrosporioides* (Sherti)

(9) *Fusarium fusarioides* (Frag. & Cif) Booth

(10) *Geotrichum candidum* Lin

(11) *Myrothecium rodium* Tode ex Fr.

(12) *Stachybotrytis atra* Corda

(13) *Trichoderma viride-2 isolate-4, CMI,* New, Survey England

(14) *Trichoderma viride-3*

(15) *Trichoderma viride-4*

(16) *Trichoderma hamatum*

(17) *Penicillium javanicum* Van eyma

In the present investigation, biological control of seed borne and soil borne diseases and crops plants was undertaken. The following aspects were taken for the present study.

1. *In vitro* testing of antagonists against five pathogenic *Fusarium* sp. viz. *Fusarium oxysporum, F. solani, F. acuminatum, F. semitectum* and *F. moniliforme.*
2. Testing selected antagonists through seed treatment and soil application.
3. Studying the mode of action of antagonists against *Fusarium* sp.
4. Studying the effect of plant extracts on the growth of the pathogens.

The antagonistic effect of fungi against five pathogenic *Fusarium* sp. was assessed by dual culture method on PDA medium (Dennis and Webster, 1971c). A 9 mm disc of antagonist was placed at one end of the petridish over the PDA medium and incubated for 48 h at room temperature (28 ± 2°C). Just opposite to the antagonist a 9 mm disc of pathogen was placed at 48 h. The zone of inhibition was measured between antagonist and pathogen after 24, 48 and 72 h. From these the most effective fungal antagonists were selected for further studies.

Spores of above antagonists were harvested from the PDA medium after 14 days of incubation period. The spores were suspended in sterile distilled water, blended and filtered through a muslin cloth. The filtrate containing conidia was centrifuged at 3000 rpm for 10 min. The supernatant was discarded and the conidial pellet was resuspended in sterile distilled water. The process was repeated once again and finally the conidia were suspended in 10 ml of 0.1 percent carboxy

methyl cellulose solution. The spore concentration of the suspension was adjusted to 4.5 to 5.8 x 10^9 conidia per ml using a haemocytometer.

Treated seeds were sown in infested soil. Each treatment was replicated three times and the pots were uniformly irrigated daily.

The antagonists effective under *in vitro* condition were used for the seed treatment in roll towel method. Seeds treated with cercobin were used for comparison. Suitable controls were maintained. The seeds were allowed to germinate at 25 ± 5°C temperature and 95 ± 2°C percent relative humidity in the germination room. The percentage of germination, shoot length, root length and dry matter production of seedlings were recorded after 14 days. Vigour indices were also calculated by following the procedure suggested by Abdul Baki and Anderson, (1973).

Cultures of above antagonists were multiplied on wheat bran peat soil medium (Sivan *et al.*, 1984). This medium was prepared by mixing wheat bran and peat soil at the ratio of 1:1 (v/v), moistened up to 40 percent level and filled in 500 ml Ehrlenmeyer flasks. They were sterilized in an autoclave for 1 h at 1.41 kg/cm^2 pressure for three successive days. This medium was inoculated with a 9 mm disc of above antagonists. The flasks were incubated at room temperature for 14 days. The inoculum was mixed with the soil at the rate of 5 g per kg of soil.

Antagonists were inoculated in the soil five days prior to the addition of pathogen inoculum and the pathogen inoculum was inoculated one day prior to seed sowing.

Result observed the table No.8 that *Trichoderma viride* checked the all ive species of *Fusarium* colonies but *T. viride-2* and *T. viride-3* were moderately effective four species of *Fusarium* except *Fusarium acuminatum*, but no, effect observed other antagonists.

Effect of Leaf-Extract of Some plants on Spore-germination

Under an exclusive study attempt has been done to use biological products to control the disease caused by *Fusarium*. Leaves of several plants have been reported to possess constituents toxic to various microoganism. These infect serve as a chemical protective barrier to infection in nature. Shekhawat and Prasad (1971) experimented with leaf-extract of *Melia azadirachta, Ocimum sanctum* and *Allium sativum* against 41 species of the pathogenic fungi. They found that *Curbularia penniseli* and *Helminthosporium* species failed to germinate, in leaf-extract of either *Melia* or *Ocimum*. Khanna and Chandra (1972) have reported antifungal properties of some plant extract against *Alternaria alternata* causing leaf-spot diseases of wheat. Mishra et al. (1974) reported complete inhibition of spore germination of *Curvularia lunata* and *Helminthosporium graminicola* in the leaf-extract of *Melia* and *Ocimum* respectively.

Fungal spores are known to be more sensitive to environment than mycelium, hence it was considered necessary to investigate the effect of leaf extracts of some medicinal plants on the germination of spores of present fungi.

To study the effect of these leaf extracts on spore germination of *F. oxysporum, F. solani* and *F. acuminatum, F.* moniliforme and *F. semitectum,* the

filtrates were centrifuged for 1/2 hour at 2000 rpm. The extracts were diluted to 50%, 100% and Hoffman's (1860) method was followed for the study of spore germination. Results on the spore germination were recorded after 24 hours of the treatement and are presented in Table 8.

It is evident from the table that out of five medicinal plant tried leaf-extract of Neem garlic and onion at 100% concentration completely checked the spore germination all the five Fusaria. In comparison to the above leaf-extract spore germination of *F. solani, F. oxysporum, F. moniliforme, F. acuminatum* and *F. semitectum* was 95, 95, 96, 92 and 95 respectively in control sets (distilled water).

Spore suspensions were made in the supernatant concentrations viz. 100% and 50% and the percentage germination of the spores was recorded after their initial time (after 5 hours of the treatment) of germination. Result obtained presented in the Table 9.

Leaf-extracts of some medicinal plants yielded satisfactory results in the present study. Leaf-extracts of *Stychnos nuxvomica, Allium cepa, Azadirachta indica, Ocimum sanctum* and *Allium sativum* at 100 percent concentration completely inhibited the spore germination of all the 5 Fusaria, six plant species were screened for their antifungal properties.

Table : 8

Effect of antagonists on pathogenic *Fusarium* population per gram soil

S. No.	Name of antogonists	Pathogenic Fusaria (No. of colonies per gram of soil)				
		Fusarium oxysporum	*F. solani*	*F. semitectum*	*F. moniliformae*	*F. acuminatum*
1.	*Aspergillus flavus*	51	48	52	47	45
	Alternaria alterna	49	49	50	44	48
	Chaetomium globosum	42	41	47	48	50
	Chaetomium arcuatum	50	45	49	51	53
	Colletotrichum state of Glomerella cingulata	55	58	57	56	54
	Cladosporium oxysporum	60	61	59	52	62
	Curvularia lunata	57	60	56	61	58
	Fusarium arthosporiodes	61	57	60	56	53
	Fusarium fasarioides	59	59	57	49	45
	Geotrichum candidum	48	48	43	45	35
	Myrothecium rodium	52	54	49	58	49
	Stachybotrys atra	49	51	53	52	48
	Trichoderma viride-2	03	07	05	08	00
	Trichoderma viride-3	00	00	00	00	00
	Trichoderma viride-4	01	04	02	05	00
	Trichoderma hamatum	04	05	06	04	01
	Penicillium javanicum	51	48	50	47	49

Result observed the above table that *Trichoderma viride* checked the all five species of *Fusarium* colonies but *Trichoderma viride-2* and *Trichoderma viride-3* were moderately effective for four species of *Fusarium* except *Fusarium acuminatum*, but no effect observed other antagonists.

Table : 9

Showing effect of leaf-extract of various medicinal plants on spore germination of *Fusarium*

S. No.	Leaf extract	Concentrations in %	Percentage spore germination				
			F. oxysporum	*F. solani*	*F. moniliformae*	*F. acuminatium*	*F. semitectum*
1.	*Strychnos nuxvomia*	50	66	76	66.07	63.19	83.07
		100	35	30	44.6	31.51	44.6
2.	*Alium cepa*	50	22.6	18.6	20.66	20.7	25.98
		100	0.0	0.0	0.0	0.0	0.0
3.	*Azadirachta indica*	50	20.5	10.50	25.07	19.06	20.0
		100	0.0	0.0	0.0	0.0	0.0
4.	*Ocimum sanctum*	50	70.6	6.67	58.6	55.6	57.8
		100	35.46	36.5	32.6	36.5	35.5
5.	*Allium sativum*	50	16.7	5.6	10.0	15.8	15.87
		100	0.0	0.0	0.0	0.0	0.0
6.	Control	--	95	95	96	92	95

Leaf-extracts of some medicinal plants yielded satisfactory results in the present study. Leaf-extracts of *Strychnos nuxvomica, Allium cepa, Azadirachta indica, Ocimum sanctum* and *Allium sativum* at 100 percent concentration completely inhibited the spore germination of all the 5 fusaria, six plant species were screened for their antifungal properties.

DISCUSSION

"Men are never so likely to settle a question rightly as when they discuss if freely".

— Thomas Babington, Lord Macaulay

Fusarium is well adapted to habitation on this earth and is present in our soil, water, air and organic materials. Perhaps more is known about the genus *Fusarium* and its species than is known about other genera of fungi.

The genus *Fusarium* may have been one of the earlier fungi to become established on earth. Structures resembling chlamydospores of *Fusarium* in coal which was estimated to date back 200 million years. But certainly if any fungus were to emigrate to another planet. *Fusarium*, as a most adaptable, versatile and pioneering genus, would be a good candidate for establishment out there. Some of its species do well with very little oxygen or water and with unusual mixtures of gases and chemicals, and flourish under extremes of environmental stress.

Fusaria are present in soils of both cultivated and non-cultivated fields and some of them are well known as wilt causing pathogens. In the present study isolations from different fields of Allahabad, Azamgarh, Ambedker Nagar, Ghazipur and Mau regions carrying fruits, trees and crop plants constantly yielded species of *Fusarium* besides a number of other fungi. Since Fusaria are well

known as wilt causing pathogens, ecological factors governing their distribution selected fields of Allahabad, Azamgarh, Ambedker Nagar, Ghazipur and Mau were studied. The soil samples from the above fields were collected monthly for one complete year. The data revealed much valuation through the three years but no significant difference was noted in number of colonies isolated from different areas in a particular month. The soils of all the selected fields were found to be sandy loam (Gangetic alluvial). It was observed that soil of fields contain maximum number of Fusaria during October and it was interesting to note that the soil conditions as well as climatic conditions were optimum and comparatively better than the other months.

Soil is a resting place for a wilt pathogens and it is primary source of inoculum for on set of plant diseases. Our knowledge regarding factors affecting survival of various wilt and root-rot causing organisms is, however, very meagre. A number of soil factors like temperature, moisture and amendment affect the population of pathogen and thus regulate the severity of infection. Creating unfavourable conditions for the pathogens either by regulating the temperature, soil moisture and by amending the soil with chemicals will help in controlling many diseases. The present study, dealing with the effect of some factors on the survival of species of *Fusarium* in soil, indicated that lowering or raising the temperature of soil or by increasing the soil moisture, the wilt of Linseed, Pea, Arhar, mango and guava can be controlled to some extent. The population of

Fusaria was considerably reduced at low or high temperature and high soil moisture. As it has been suggested by orton (1953), Kapoor (1954) and Dhingra and Sinclair (1975). The reduction of *Fusarium* species population at high soil moisture level may be attributed to increased bacterial activity in turn may result in the lysis and the digestion of fungal mycelium and spores.

The number of fungi decreased with increase in the soil depths. The reduction in number of fungi with increasing soil depth may be due to the reduction in organic matter and oxygen, and increased carbon dioxide. *Pencillia, Trichoderma,* Aspergilli and *Fusaria* seem to tolerate the fluctuations in environmental conditions as they were found almost in all the soil samplings in the present study. They have been reported to be most tolerant ones to the adverse conditions (Rai *et al.*, 1970; Dubost, 1962; Venkataraman and Raiyalakshmi, 1971; Phanasenko, 1967). Hence it may be concluded that these antagonists have wide ecological spectrum. The dominance of *Pencillia, Aspergilli, Fusaria* and *Trichoderma* in the present study agrees with the reports of Chrinstensen *et al.* (1962), Kamal and Gupta (1973) and Upadhyay and Rai (1979).

Generally the population of micro-organisms declines with an increase in depth of soil profile. The root system of plants favours the growth of microbes by excretion of nutrient substances (Krasilnikov, 1958). Similar plea about the distribution of micro-organisms at various depth has been given by Jayasheela and Oblisami (1975) but they reported that the rhizosphere effect increased with

increase in depth. The population of these micro-organisms differs quantitatively, qualitatively (Kaiznelson et al., 1948) and physiologically (Rouatt and Katznelson, 1957).

Soil-borne fungi/fungal pathogens are influenced by soil water factor which is an important factor for better growth and survival of such fungi (Cook and Pependick, 1970). The propagules of saprophytic and pathogenic fungi survive in the root region of plants in adverse conditions by colonizing organic debris and producing chlamydospores and sclerotia (Garrett, 1966).

A number of *Fusaria* were found from the soil of favious fields and studies on their morphological characters indicated that they were isolates of *F. solani, F. oxysporum, F. acuminatum, F. moniliforme* and *F. semitectum.* Pathogenicity test of these isolates revealed that all of the five species caused wilting of the above crop and fruit plants. The incidence of wilting in relation to different soil sand composition was studied and the highest percentage of wilting was observed in the pots containing sand only by all the pathogens, while the lowest in pots containing soil only.

The seeds after harvest became infected through pods and the infection involved the seed-coat and usually considerable parts of the cotyledons. The site of infection was the fleshy cotylendons, which provided a rich nutritious base for the

seedling as well as for the pathogens and possibly accounted for the important role of seed transmission of these pathogens.

Fusarium sp. provided examples of serious seed-borne fungi that penetrated from the vascular system through the funiculus into the seed. Rudolph and Harrison, (1945); isolated *F. moniliforme, F. oxysporum* and *F. acuminatum, F. solani, F. semitectum* from vascular bundles in all parts of cotton including the internal tissues of the seed. Vidhyasekaran et al., (1970); detected *Fusarium moniliforme* in the embryo of 80 percent of the stored rice. Mathur and Arya and Sharma, (1962). According to Klisiewicz, (1963); *Fusarium oxysporum* spreaded intra and intercellularly in the tissues of the pericarp and seed coat of safflower.

The invasion of fungi at different stages during development of the seed primordia and maturing seed was of great importance to the fate of the seed. If invasion took place when the seed was nearing full maturity, only the seed coat, or pericarp; functioning as seed coat was invaded, often only the epidermal layers became involved. The parasite existed as dormant mycelium in the seed-coat or its conidia contaminated the surface of the seeds. The fungus was parasitic on the weakened tissues.

In spite of the recent extensive studies by several investigators on physiology, pathology and biochemistry of the pathogenic fungi, the point of convergence regarding their general behaviour has not been reached so far. A

sailent land mark of such studies has been the apparent diversity of responses manifested by organisms to the compounds encountered in nature. A comparative knowledge of the subject might be of some aid in understanding the relationship between the host and the parasite, and then we may control or minimise the diseases.

Studies on pathogenicity of five species of *Fusarium* indicated that *F. oxysporum* was most pathogenic as it could cause high percentage of wilting in the Linseed, Pea, Arhar, mango and guava seedlings. They were also capable of infecting a wide range of crop plant. *Fusarium* were capable of causing wilt of Arhar, Linseed, Pea, Mango and Guava seedlings. The wilting was characterised by gradual withering, yellowing and drying of leaves. Later on it was followed by drying of entire seedlings. It was observed that there was slightly higher percentage of infection in case of injured root seedlings than those uninjured. Out of the five species *F. oxysporum* was more pathogenic to Arhar, Linseed, Pea, Mango and Guava seedlings than the other two species, as it caused a higher percentage of wilting in the seedlings.

Investigations on the extracellular production of toxins *in vitro* revealed that *F. oxysporum* was more efficacious in producing the toxins, as its culture filtrate could cause cent percent wilting of seedlings within 4 days. It was also interesting to note that *F. oxysporum* produced a high amount of Fusaric acid *in vitro*. Thus,

the severity of infection caused by *F. oxysporum* may be correlated with its capacity to produce high amount of Fusaric acid.

Isolation studies of mycoflora from soil, seeds and infected plant material produced some interesting results. In all five Fusarium sp. were isolated. There was not much variation seen in the fungal flora of seeds which were collected from different places although their frequencies were different in each sample.

A number of reports have appeared in the literature (Cochrane and Conn., 1950; Gordon, 1950; Tandon and Bilgrami, 1957; Chandra, 1961; Tandon and Srivastava, 1963) regarding the toxic effect of nitrite nitrogen on various fungi. The toxicity has been found to be closely related to hydrogen-ion concentration of the medium. In the present study also, the sodium nitrite was toxic (at lower ph) to growth of all the *Fusarium* species. Thus it may be possible to control *Fusarium* population in acidic soil addition of sodium nitrite.

Results from another set, where the seedlings were kept in culture filtrates of *Fusarium* species, showed that seedlings wilted within seven days. All the seedlings kept in culture filtrates of *F. oxysporum*, *F. solani*, *F. acuminatum*, wilted while in *F. semitectum* and *F. moniliforme* the percentage was a little less. Controls remained healthy in both set of experiments.

Basically the wilt diseases are associated with plants that contain vessels in their xylem, that is, the angiosperms. While wilting, or loss of turgidity, is a

common symptom of disease, it may result from one of many possible causes. The vascular wilts are set off as a group because of the association of symptoms with the presence of the pathogen in the vascular system of the host, usually in the xylem tissue. The most common pathogens are vascular *Fusaria.*

Two theories put forward to explain the phenomenon of wilting. The first theory is called the "toxin theory" and the second the "plugging theory" or "vascular occlusion theory". The toxin theory is based on the fact that the permeability of parenchymatous cells in leaves increases so that they lose their osmotic efficiency and this results in wilting, Gaumann (1951, 1954, 1957).

When there is very high resistance to the flow of water through the xylem, the leaves receive less water than they transpire and as a consequence, they wilt. This is the basis of the plugging theory, Ludwig (1952).

Diseases produced by the pathogenic *Fusaria*, they are Wilt of pigeon pea., Arhar, Linseed, Pea, Mango and Guava. The wilting is characterized by gradual, sometimes sudden, yellowing withering and drying of leaves followed by the drying of the entire plant or some of its branches. Wilt of Linseed, the wilt disease of flax or linseed plant is very widespread and occurs in almost all the countries where linseed is grown. Wilt of cotton, in India, the disease was first reported in Nagpur in 1908. The disease has now spread to almost all the black cotton soil tracts of the country. The pathogen causes extensive damage in the heavy soils and

is more or less absent in the loamy soils of the northern plains and the Indo Gangetic plains. Panama disease of Banana, the disease is widespread throughout Africa, Asia, Australia and Oceania, West Indies and North, Central and South Africa. Fusarium diseases of Beans, Peas and Lentils, Beans (*Phaseolus vulgaris*), peas (*Pisum sativum*), and Lentil (*Lens esculenta*) are among the most important and widely grown food crops in the leguminosae. Bean Root Rot, the disease was first described by Burkholder in western New York State in 1916. Pea Wilt, *Fusarium* wilt of peas, caused by *Fusarium oxysporum* f. sp. *pisi* race 1, was first reported in Wisconsin in 1924 Jones et al., (1925).

Some of the important contributions in this area are by Gaumann (1954), Dimond and Waggoner (1953) on vivotoxins; host specific plant toxins have been discussed by Pringle and Scheffer (1964); and toxins and plant diseases have been discussed by Kalyansundram and Charudattan (1966) and Husain and Janardhan (1976).

De Bary (1886) showed that plant pathogens produce toxins but experimental evidence to support this hypothesis was available only in 1913 (Hutchinson,1913). The credit for the discovery of toxin is generally given to Roux and Yersin (1888) who first visualized the presence of a toxin in the disease caused by *Corynebacterium diptheriae*.

Toxins produced by *Fusarium* : Lycomarasmin, one of the earliest substances to be implicated in wilt diseases was the dipeptide lycomarasmin Gaumann, (1951).

Davis (1969) first found the toxin in flax, tomato, and watermelon 3 or 7 days after inoculation with their particular formae. Trione (1960) found that *F. oxysporum* f. sp. *lini* produced Fusaric acid on susceptible but not resistant flax stems sterilized with propylene oxide and therefore killed.

For many *Fusaria*, survival in soil depends on chlamydospores that have the ability to withstand adverse environmental conditions, Nash et al., (1961). The possible role of inorganic salts in stimulating chlamydospore formation in conidia was studied by Hsu and Lockwood (1973).

In contrast to the effects of carbon sources, the reported effects of nitrogen on chlamydospore formation are contradictory. At low macroconidial densities in salt solutions, inorganic nitrogen did not inhibit chlamydospore formation in *F. solani* f. sp. *radicicola* and *F. solani* f. sp. *phaseoli*, Griffin (1976).

The present study deals with some factors affective survival of five species of *Fusarium* viz., *Fusarium solani, F. oxysporum, F. acuminatum, F. semitectum* and *F. moniliforme*, isolated from soils of different places.

However, many workers including Garrett (1944, 1956, 1963, 1970), Butler (1953), Stover (1953 a, b), Papavizas and Davey (1961), Sen Gupta and Roy

(1971) as well as Dhingra and Sinclair (1976) have extensively reported the saprophytic behaviour of fungi in soil.

In the present study of Allahabad district was selected for survival studies. The soil was dried, and sieved and was infested with 3% maize meal sand culture.

Temperature is an important environmental factor affecting the metabolic activities of the fungi. It is evident from the study that growth, sporulation and chlamydospore formation of the present organisms were prominently influenced by temperature variation.

The maximum survival of all the *Fusarium* species was observed between 20°C and 25°C. Their number (colonies) decreased as the temperature was either increased or decreased. Maximum number of colonies of *F. acuminatum, F. oxysporum, F. solani* were observed at 25°C while in case of *F. semitectum* and *F. moniliforme* maximum number of colonies retrieved was at 20°C. At 5°C and 40°C the survival of the present fungi was minimum.

In the present investigation the effect of soil moisture on the survival of five species of *Fusarium* was assayed in soil in which the water holding capacity was adjusted at different percentage ranging from 10 to 100 percent. The survival of the present *Fusarium* species was maximum when soil moisture was maintained at 30% water holding capacity.

The present study indicated that the five species of *Fusarium* were strongly aerobic and their population can be reduced by maintaining the soil in a saturated condition. This view is also supported by Stover (1953 a).

Soil samples were amended with glucose, sucrose and starch. The different carbon sources were added at a rate equivalent in carbon to that provided by 1% glucose in air dried soil (w/w). Non-amended soils served as control. All soil samples were then adjusted to 30% water holding capacity of the soil on an ovendry weight basis. Samples were incubated at 22°C ± 1°C for 4 weeks.

Biological interference with epidemics has been dealt with by Darpoux (1960). The first successful control of a root disease by biological means was by Millard and Taylor (1927) who reported control of scab in potatoes caused by *Streptomyces scabies* by green manuring.

Biological control must work within the context of biological balance. The soil is more stable than the aerial environment in practically all respects, but it can be slowly altered by many conditions.

The studies discussed in this section suggest that chemicals may achieve disease control by nudging the microbiological balance rather than directly killing the pathogen. This could open a new era of plant disease control, even as sulfanilamide and penicillin changed medical practice from killing human pathogens with poisons to inhibiting them with antibiotics.

Biological control by a single introduced microorganism may be successfully used when the soil or plant surface is virtually free of other microorganisms. Some success also has been attained by inoculating nonsterile plant propagules with antagonists.

Vascular pathogens that have breached the host defenses and reached the vascular elements are well-protected from antagonists.*Pseudomonas syringae* produces toxins *in-vitro* that are inhibitory to *Ceratocystis ulmi,* the cause of Dutch elm disease, Myers et al., (1978).

Under an exclusive study attempt has been done to use biological products to control the disease caused by *Fusarium.* Leaves of several plants have been reported to possess constituents toxic to various microoganism. These infect serve as a chemical protective barrier to infection in nature. Shekhawat and Prasad (1971) experimented with leaf-extract of *Melia azadirachta, Ocimum sanctum* and *Allium sativum* against 41 species of the pathogenic fungi.

Spore suspension were made in the supernatent concentrations viz. 100% and 50% and the percentage germination of the spores was recorded after their initial time (after 5 hours of the treatment) of germination.

Antagonists like *T. viride-2, T. hamatum* and *T. viride-3* exhibited greater antagonism and they differed in their mechanism of action against the pathogen. *T. viride-2* and *T. viride-3* were found to overgrow. *Fusarium* sp., *T. hamatum*

secreted yellowish metabolites which destroyed the growth of the pathogen at the zone of contact. Parasitation of antagonist was also reported by Vesely (1978b).

Seed treatment with *T. viride* was found to be as effective as fungicidal treatment that the *T. viride-2* and *T. hamatum* treated seeds were as effective as fungicides tested.

Soil inoculation with *T. viride-2 effectively controlled the wilt and seed rots followed by* T. viride-2, T. viride-4 and *T. viride-3.* Among the fungal antagonists *T. viride-2* was found to be superior in reducing the wilt.

The application of leaf extract in controlling the plant diseases could be a good alternative to chemical fungicides and pesticides. These products are biodegradable therefore do not alter the soil physio-chemical characteristics. In some cases these plant product could be freely advised to apply during the storage of the grains and fruits as their residue if enters in our body through food acts as a medicine and not as a toxin.

Leaf-extracts of some medicinal plants yielded satisfactory results in the present study. Leaf-extracts of *Strychnos nuxvomica, Allium cepa, Azadirachta indica, Ocimum sanctum* and *Allium sativum* at 100 percent concentration completely inhibited the spore germination of all the 5 Fusaria, six plant species were screened for their antifungal properties.

CHAPTER – 10

SUMMARY

SUMMARY

A comprehensive survey of various fields of Allahabad, Azamgarh, Ambedker Nagar, Mau, Ballia, Ghazipur and its adjacent regions were made and various wilted crop plants, trees, soil and seed samples were collected. Blotter, Agar Plate and Blotter Roll methods, were used for isolation of Fusarium species. Fusaria were isolated, purified and maintained on Malt-extract and Potato Dextrose Agar media. Morphological studies were carried out and identifications were made.

Fusaria isolated from soil, seed samples and wilted trees at various places are as follows :

Table : 1
***Fusaria* isolated from various crops field, wilted trees and seeds**

***Fusaria* isolated from soil**	***Fusaria* isolated from seeds**	***Fusaria* isolated from wilt trees**
Allahabad District		
Fusarium oxysporum	*Fusarium. semitectum*	*Fusarium oxysporum*
F. solani	*F. acuminatum*	*F. equiseti*
F. equiseti	*F. culmorum* *	*F. lateritium*
F. acuminatum	*F. heterosporum*	*F. nivale*
F. roseum	*F. nivale*	*F. decemcellulare* *
Azamgarh District		
F. poae *	*F. solani*	*F. moniliformae*
F. oxysporum	*F. semitectum*	*F. sambucinum*
F. graminearum	*F. roseum*	*F. sulphureum* *
F. solani	*F. oxysporum*	*F. equiseti*
F. equiseti	*F. sambucinum* *	*F. oxysporum*
F. xylarioides *	*F. acuminatum*	*F. heterosporum*
F. stilboides *	*F. fusiroides*	*F. avenaceum*

Ambedker Nagar District

F. acuminatum	*F. nivale*	*F. decentaelluelare* *
F. oxysporum	*F. sumbacin* *	*F. oxysporum*
F. culmorum	*F. graminearum*	*F. roseum*
F. lateritium	*F. xylarioides*	*F. acuminatum*
F. avenaecum	*F. heterosporum*	*Fusarium sp.*
F. moniliformae	*F. oxysporum*	*Fusarium sp.*
F. solani	*F. equiseti*	*F. semitectum*

Mau District

F. oxysporum	*F. stilboides* *	*F. heterosporum*
F. equiseti	*F. literitium*	*F. moniliformae*
F. solani	*F. culmorum*	*F. oxysporum*
F. roseum	*F. decemcellulare* *	*F. acuminatum*
F. heterosporum	*F. avenaceum*	*F. solani*
F. acuminatum	*F. oxysporum*	*F. graminearum*

Ballia District

Fusarium acuminatum	*F. equiseti*	*F. sambucinum* *
F. solani	*F. juruanum* *	*F. lateritium*
F. tabacinum *	*F. arthrosporiodes*	*F. nivale* *
F. stoveri	*F. moniliformae*	*F. oxysporum*
F. buxicola *	*F. larvarum* *	*F. illudens* *
F. gigas *	*F. poae*	*F. sphaeral* *
F. concolor *	*F. epistromum* *	*F. fusaioides*
F. arthrosporioides	*F. oxysporum*	*F. semitectum*
	F. solani	

Ghazipur District

F. oxysporum	*F. heterosporum* *	*F. oxysporum*
F. equiseti	*F. gigas* *	*F. stoveri* *
F. roseum	*F. fusiroides*	*F. stilboides* *
F. poae *	*F. solani*	*F. moniliformae*
F. acuminatum	*F. acuminatum*	*F. arthrosporioides*
F. solani	*F. concolar* *	*F. acuminatum*
F. graminearum	*F. xylarioides* *	*F. lateritium* *
		F. equiseti

* *Fusarium* species were reported from the first five in related areas :

Isolation studies revealed that out of 36 species of *Fusarium*, 20 species from soil samples, 26 species from seed samples and 23 species from wilted trees were isolated.

Results from the pathogenicity tests revealed that among the *Fusaria* isolated, five species of *Fusarium* were capable of causing wilt of Arhar, Linseed, Pea, Mango and Guava seedlings. The wilting was characterised by gradual withering, yellowing and drying of leaves. Later on it was followed by drying of entire seedlings. It was observed that there was slightly higher percentage of infection in case of injured root seedlings than those uninjured. Out of the five species *F. oxysporum* was more pathogenic to Arhar, Linseed, Pea, Mango and Guava seedlings than the other two species, as it caused a higher percentage of wilting in the seedlings.

Results from another set, where the seedlings were kept in culture filtrates of *Fusarium* species, showed that seedlings wilted within seven days. All the seedlings kept in culture filtrates of *F. oxysporum, F. solani* and *F. acuminatum,* wilted while in *F. semitectum* and *F. moniliforme* the percentage was a little less. Controls remained healthy in both set of experiments. Thus from the above experiments it is clearly evident, that *F. oxysporum, F. solani* and *F. acuminatum* causes more damage than other one species of *Fusarium* and from second set that some toxic substance also plays a role in causing wilting of Arhar, Linseed, Pea, Mango and Guava seedlings. An attempt was therefore made to detect the toxic substance (Fusaric acid) in the culture filtrates of the five species of *Fusarium.*

Aqueous mycelial and spore suspensions of the organisms prepared from 7 to 10 days old cultures, were sprayed on injured as well as uninjured leaves and

stems of their suspective host plants with the help of hand automizer. Humidity was maintained by covering it with polythene bag with some sterilized water at the base. Suitable controls were maintained for each treatment. In each case similar conditions such as amount of inoculum, water and same kind of soil etc., were provided. Fungi which were found to cause root-rot stem-rot wilt and seedling blights and leaf-spots.

When the *Fusaria* were tested for its pathogenicity in pot culture experiments, the first symptom of the disease occured 15 days after sowing, when the plant was 6" to 9" in height, starting from yellowing of leaves and ultimately the plants wilted. The roots shreaded out due to decaying. Dark brown lessions were observed on the sheath or collar region of the plants.

Spore morphology is the major character in the identification of *Fusaria*. Spores may be conidia produced as simple or polyphialidic slime spores or as enteroblastic spores (i.e. a conidium produced through a pore without the involvement of the outer wall of the conidiophore and enlarging without the formation of a septum), or chlamydospores which in many species form the resting stage. Conidia may occur as 0-1 septate, pyriform, fusoid to oval microconidia through to straight or curved, 0-10 or more septate macroconidia.

Investigations on the extra-cellular production of toxins in vitro revealed that *F. oxysporum, F. solani, F. acuminatum, F. semitectum* and *F. moniliforme*

were more efficacious in producing the toxins. It was interesting to note that they produced a high amount of Fusaric acid *in vitro*.

For many *Fusaria*, survival in soil depends on chlamydospores that have the ability to withstand adverse environmental conditions. On the basis of present study we suggest defining the chlamydospore of *Fusaria*. Chlamydospore a viable, asexually produced accessory spore resulting from the structural modification of a vegetative hyphal segment or conidial cell possessing a thick wall mainly consisting of newly synthesized cell wall material; its function is primarily survival in soil.

The present study deals with some factors affective survival of five species of *Fusarium* viz., *Fusarium solani, F. oxysporum, F. acuminatum, F. semitectum* and *F. moniliforme*, isolated from soils of different places. For this Allahabad district was selected for survival studies. The soil was dried, and sieved and was infested with 3% maize meal sand culture. For the study of the effect of temperature on the survival of the present five species of *Fusarium*.

The maximum survival of all the *Fusarium* species was observed between 20°C and 25°C. Their number (colonies) decreased as the temperature was either increased or decreased. Maximum number of colonies of *F. acuminatum, F. oxysporum, F. solani* were observed at 25°C while in case of *F. semitectum* and *F. moniliforme* maximum number of colonies retrieved was at 20°C. At 5°C and 40°C

the survival of the present fungi was minimum. No colonies, however, could be recovered at 45°C.

Effect of environmental factors on disease development revealed that by lowering and raising soil temperature and with an increase in soil moisture, fusarial diseases could be controlled to some extent. This was due to reduction in fusarial population and an increase of bacteria.

In vitro tests of fungal antagonists against *Fusarium oxsysporum, F. solani* and *F. acuminatum, F. semitectum, F. moniliforme, Trichoderma viride-2, T. viride-3, T. viride-4 and T. hamatum* were highly inhibitory seedling raised from seeds treated with *T. viride-2, T. viride-3* and *T. viride-4* produced longer roots of crops under investigation seeds treated with *T. viride-2, T. viride-3, T. hamatum* and *T. viride-4*. Produced longer shoots seedlings raised from seeds treated with *T. viride-2, T. hamatum, T. viride-3, T. viride-4*. Produced higher dry matter seedlings raised from seeds treated with *T. viride-2, T. hamatum, T. viride-3* and *T. viride-4* were recorded higher vigorous.

Soil application of antagonists viz., *T. viride-2* and *T. viride-4* at 5 gm per Kg. of unsterilized sick soil reduce the incidence of wilt disease. Soil application of antagonists viz. *T. viride-2* and *T. hamatum* was also superior to other treatments in controlling the wilt disease. Culture filtrates of *T. hamatum* and *T.*

viride-2 were found to effectively inhibit the growth of the pathogen. Some plant species were screened for their antifungal properties.

Spore suspension were made in the supernatant concentrations viz. 100% and 50% and the percentage germination of the spores was recorded after their initial time (after 5 hours of the treatment) of germination.

Leaf-extracts of some medicinal plants yielded satisfactory results in the present study. Leaf-extracts of *Strychnos nux-vomica, Allium cepa, Azadirachta indica, Ocimum sanctum* and *Allium sativum* at 100 percent concentration completely inhibited the spore germination of all the 5 Fusaria, five plant species (*F. oxysporum, F. solani, F. semitectum, F. acuminatum* and *F. moniliforme*) were screened for their antifungal properties.

CHAPTER – 11

REFERENCES

REFERENCES

"In science, read by preference, the newest works; in literature, the oldest. The classic literature is always modern".

— Edward Bulwer Lytton

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